

SDS – POLYACRYLAMIDE GEL OF OSTEOINDUCTIVELY ACTIVE
PROTEINS FROM HPLC

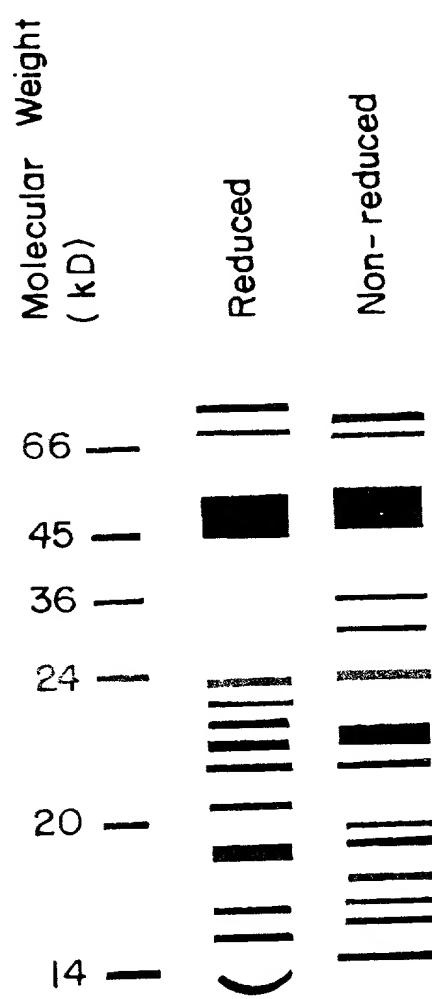


FIG. 1

FIGURE 2

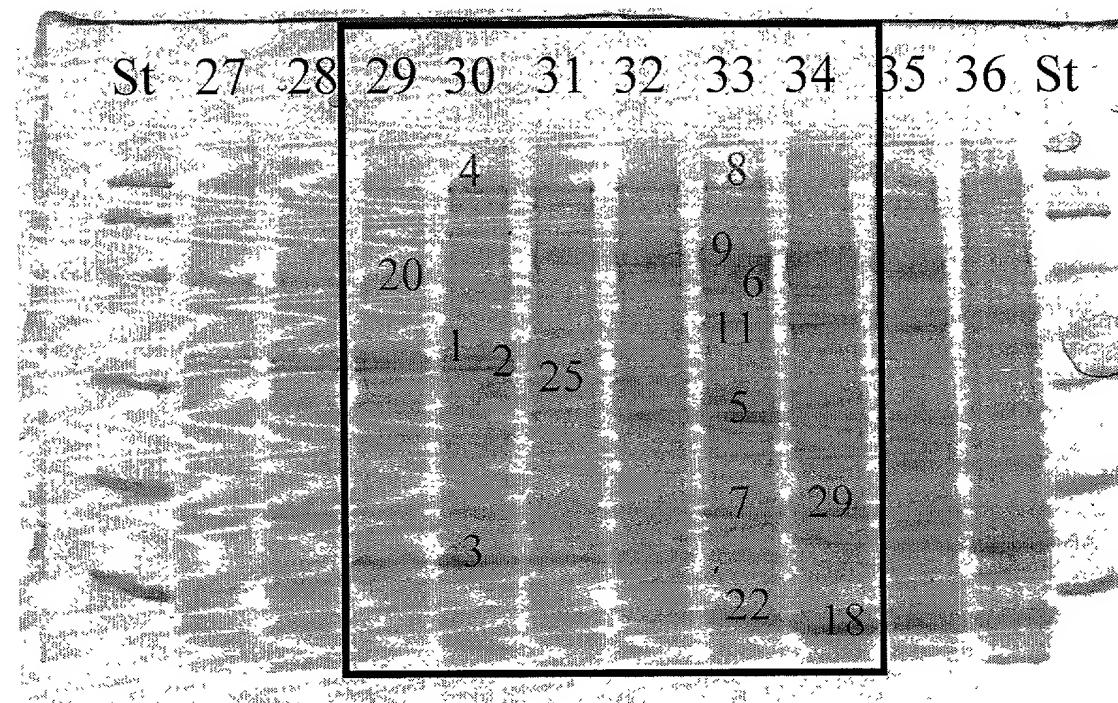
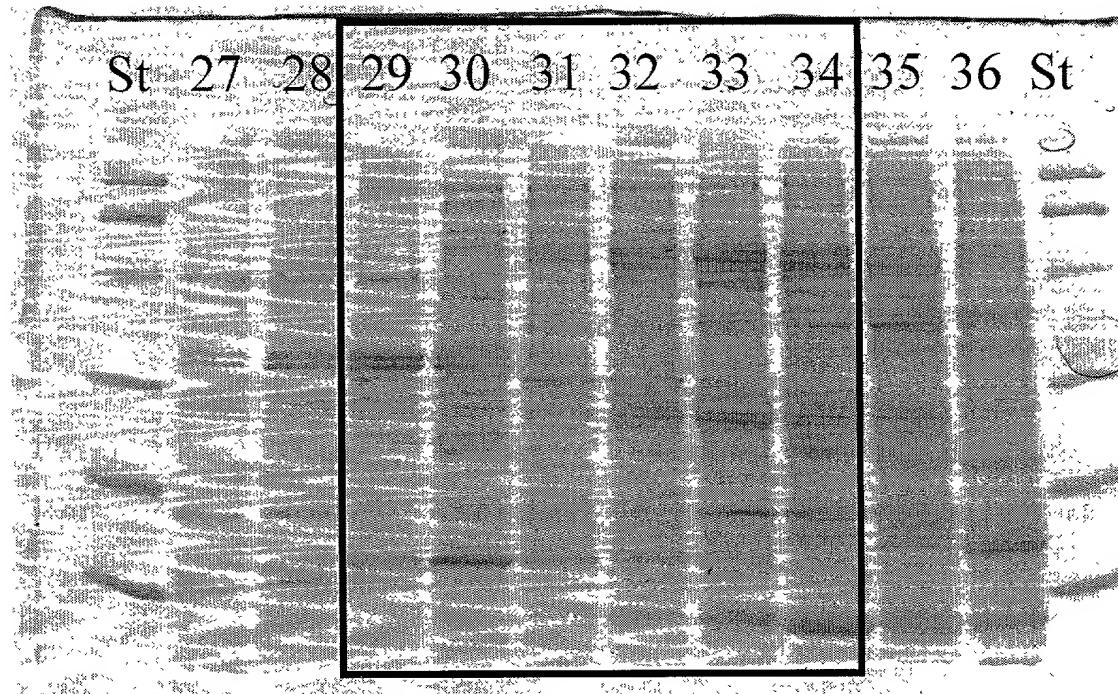
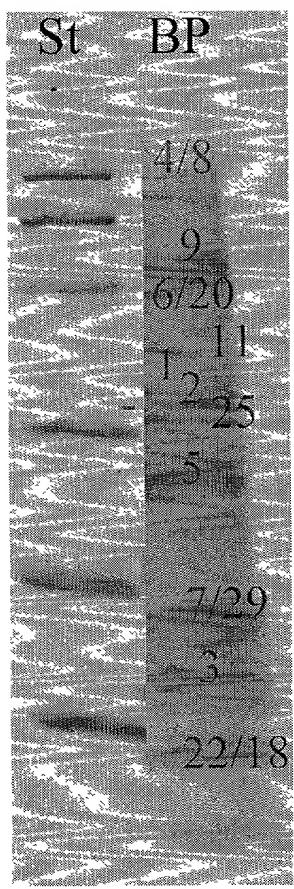


FIGURE 3



Band No.	Identity
1	Histone H1.c
2	Histone H1.c
3	Ribosomal protein RS20
4	Similar to ribosomal protein LORP
5	BMP-3
6	$\alpha 2$ macroglobulin RAP and BMP-3
7	Similar to ribosomal protein LORP
8	BMP-3
9	BMP-3
11	Ribosomal protein RL6 and BMP-3
18	TGF- $\beta 2$ / SPP 24
20	Factor H
22	TGF- $\beta 2$
25	BMP-3 and H1.x
29	BMP-3 and ribosomal protein RL32

FIGURE 4

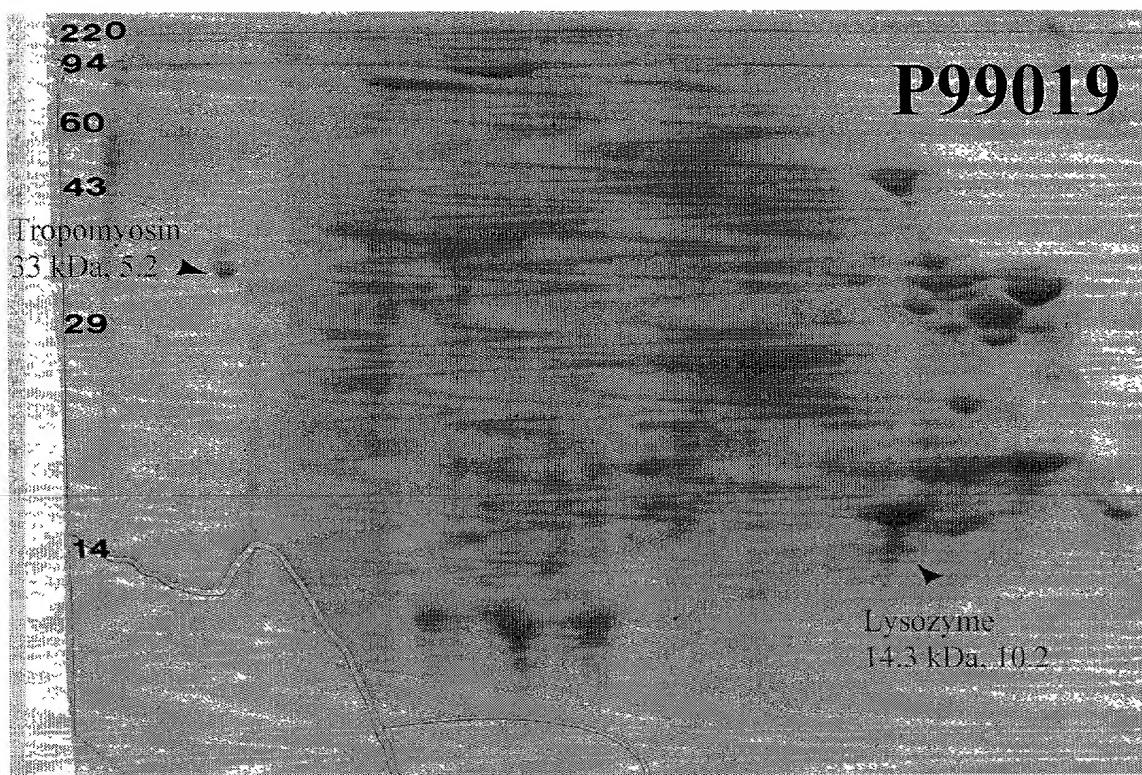


FIGURE 5

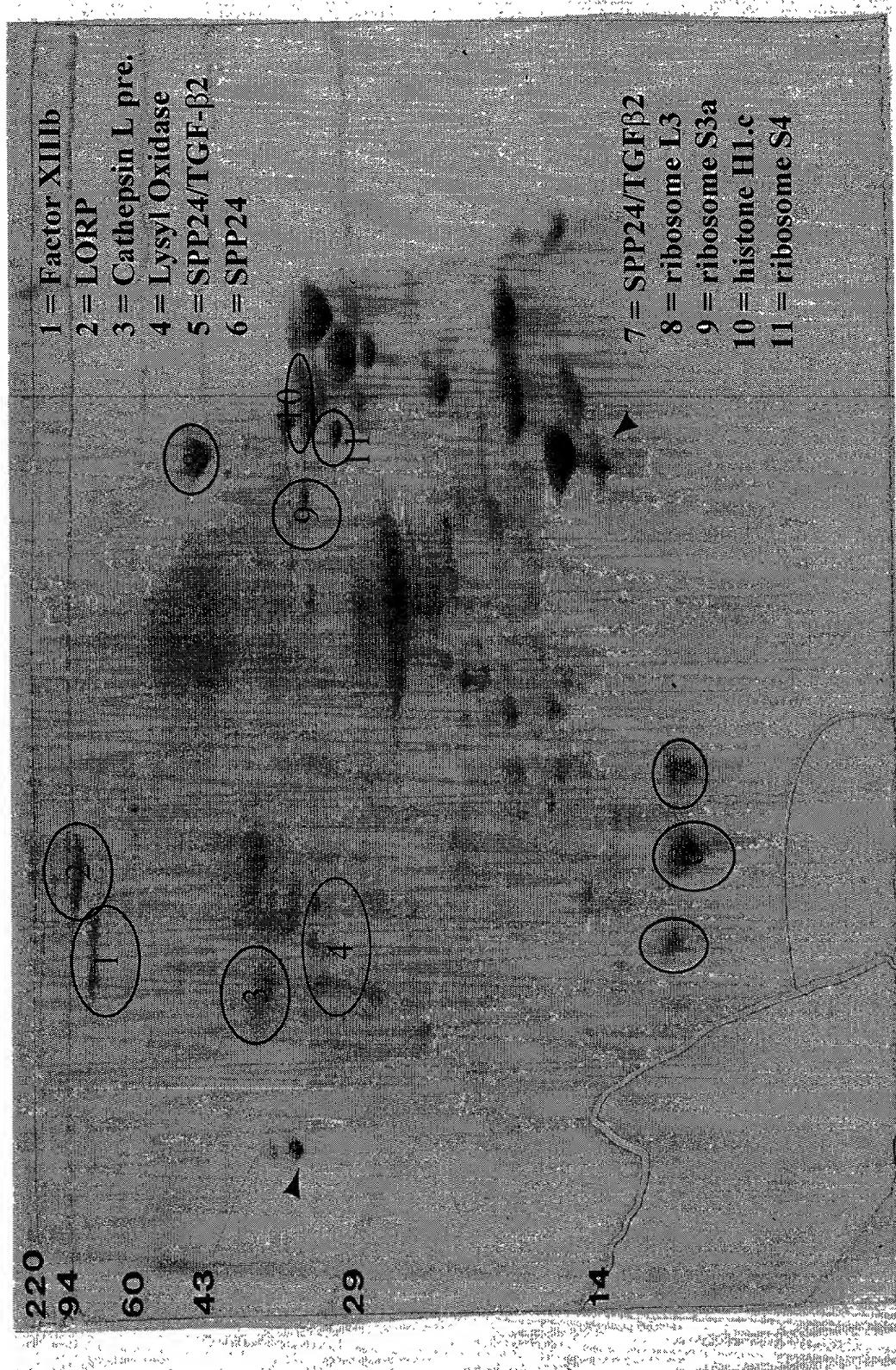


FIGURE 6

Columbia University / HHMI Protein Core

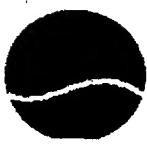
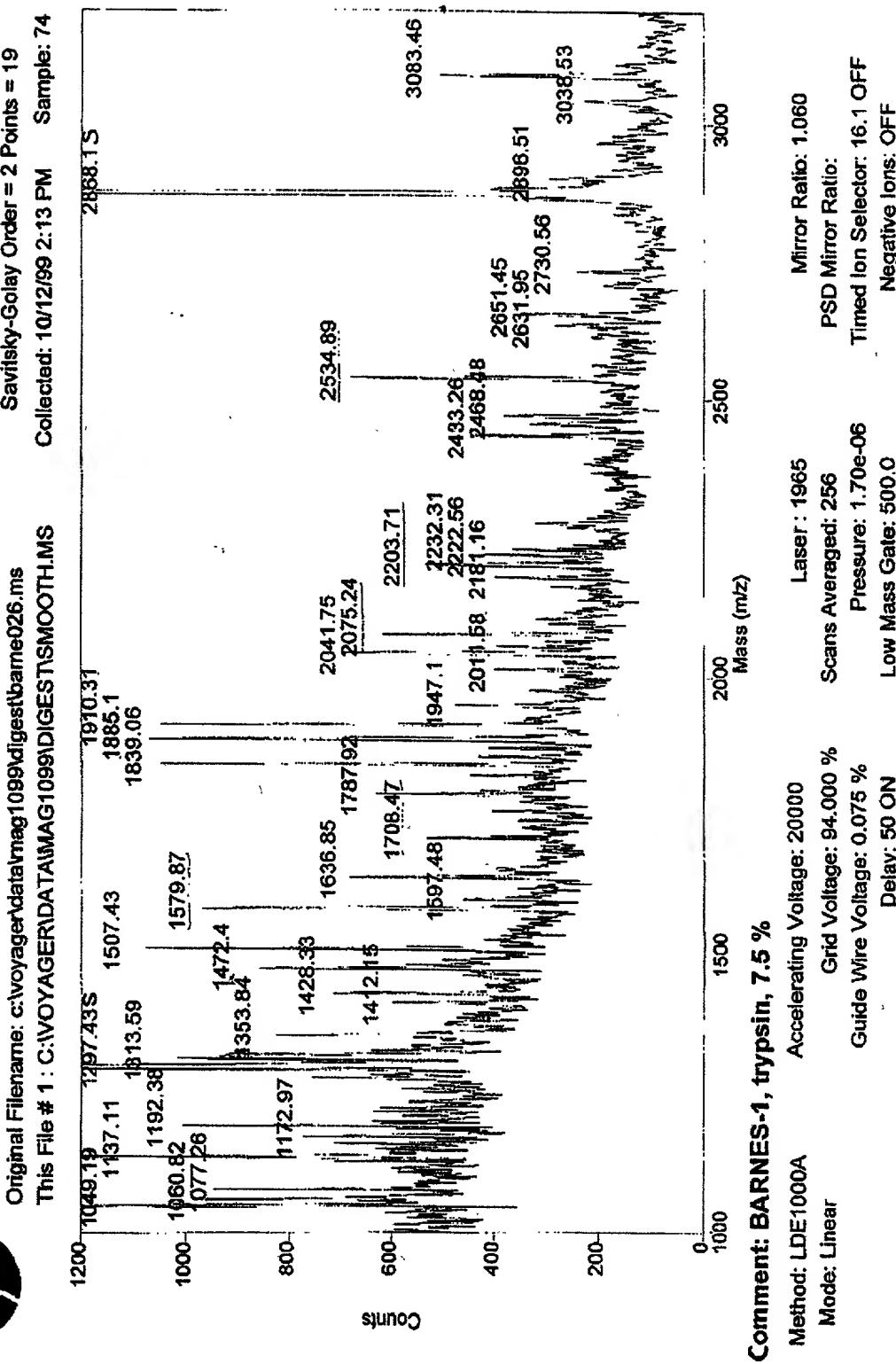


Figure 7A (Band 1)



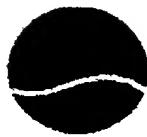
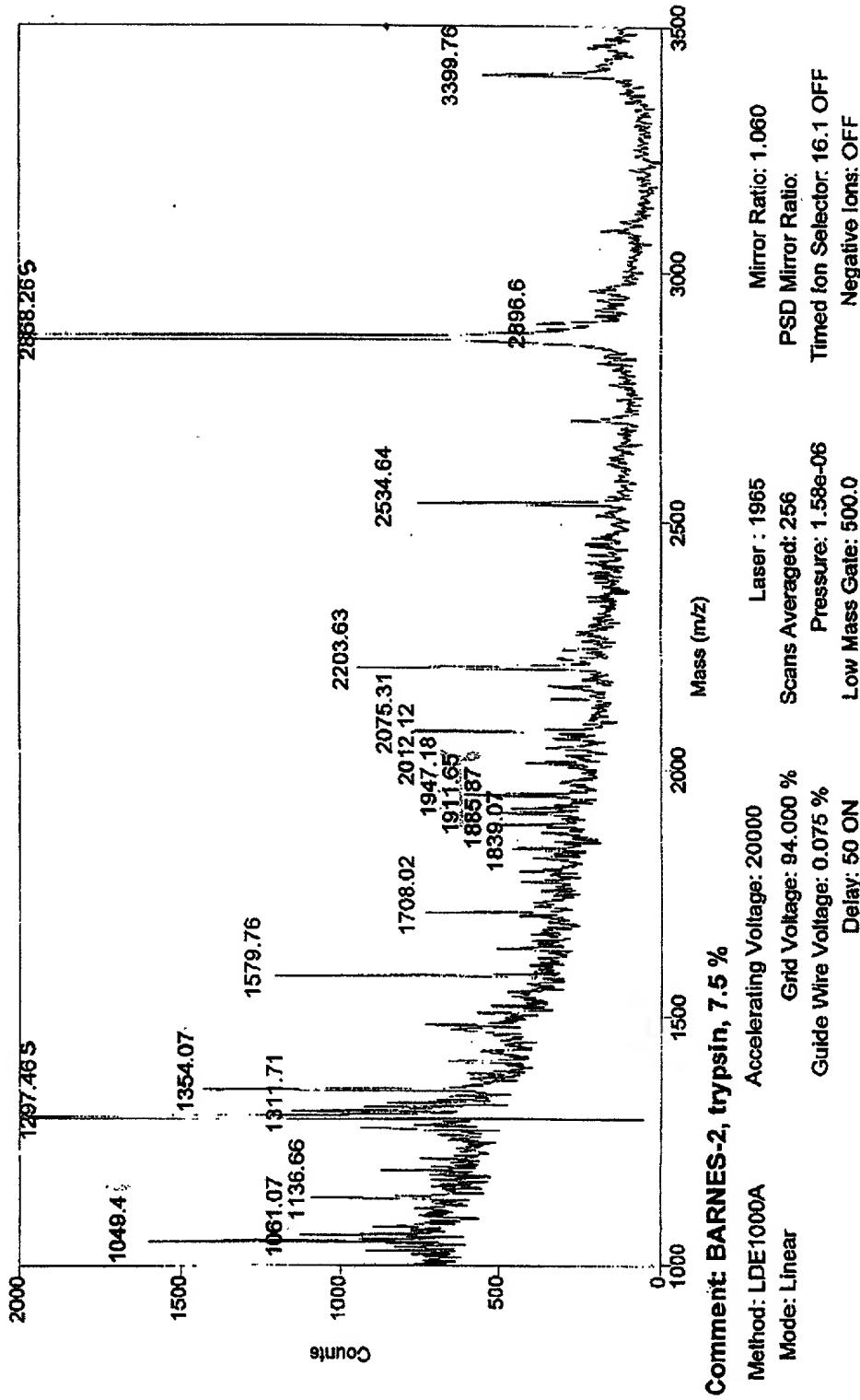


Figure 7B (Band 2)

Columbia University /HHMI Protein Core

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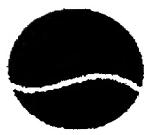


Figure 7C (Band 3)

Columbia University /HHMI Protein Core

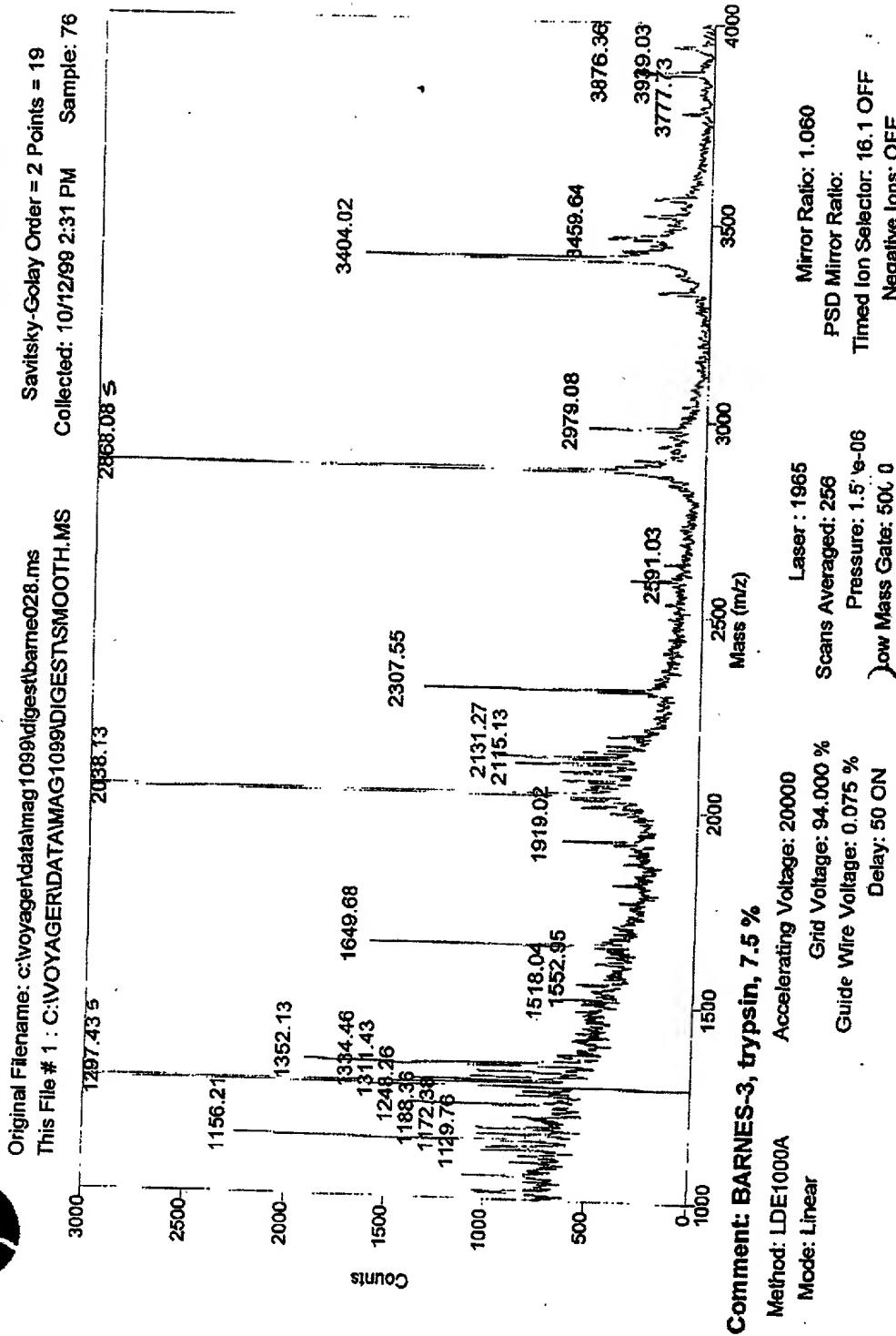


Figure 7D (Band 4)



Columbia University / FHHMI Protein Core

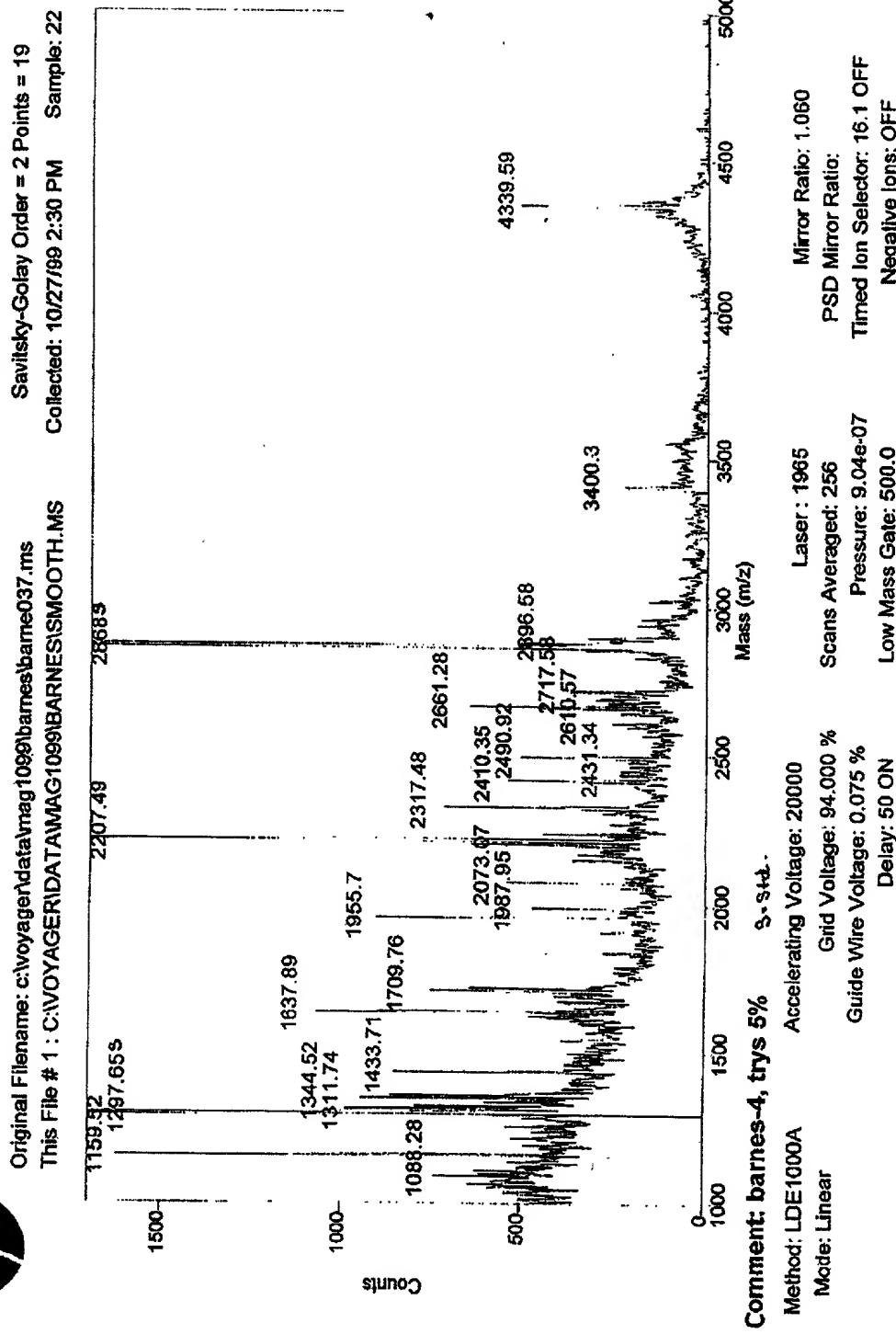
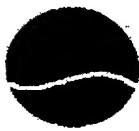
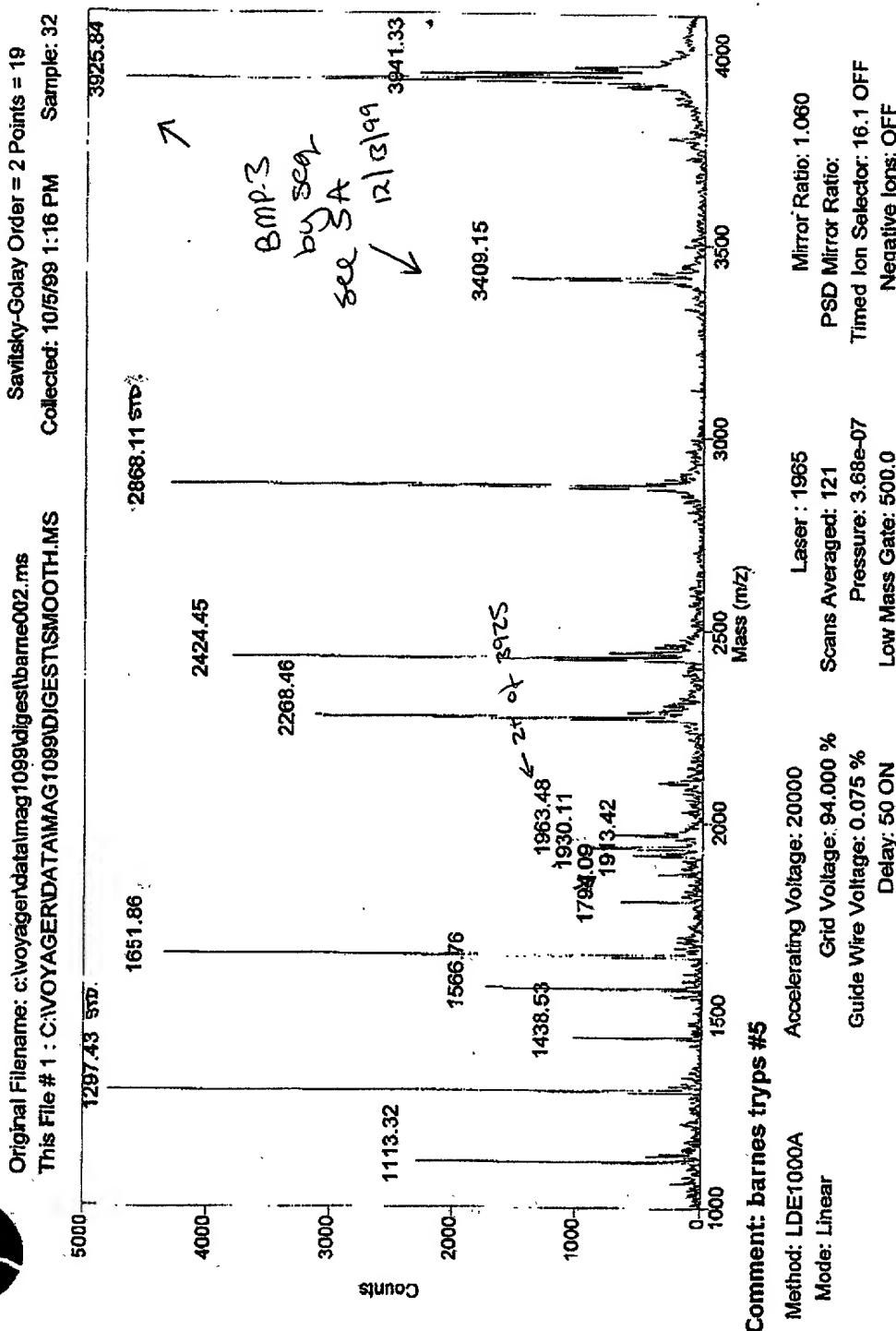


Figure 7E (Band 5)



Columbia University /HHMI Protein Core



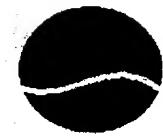
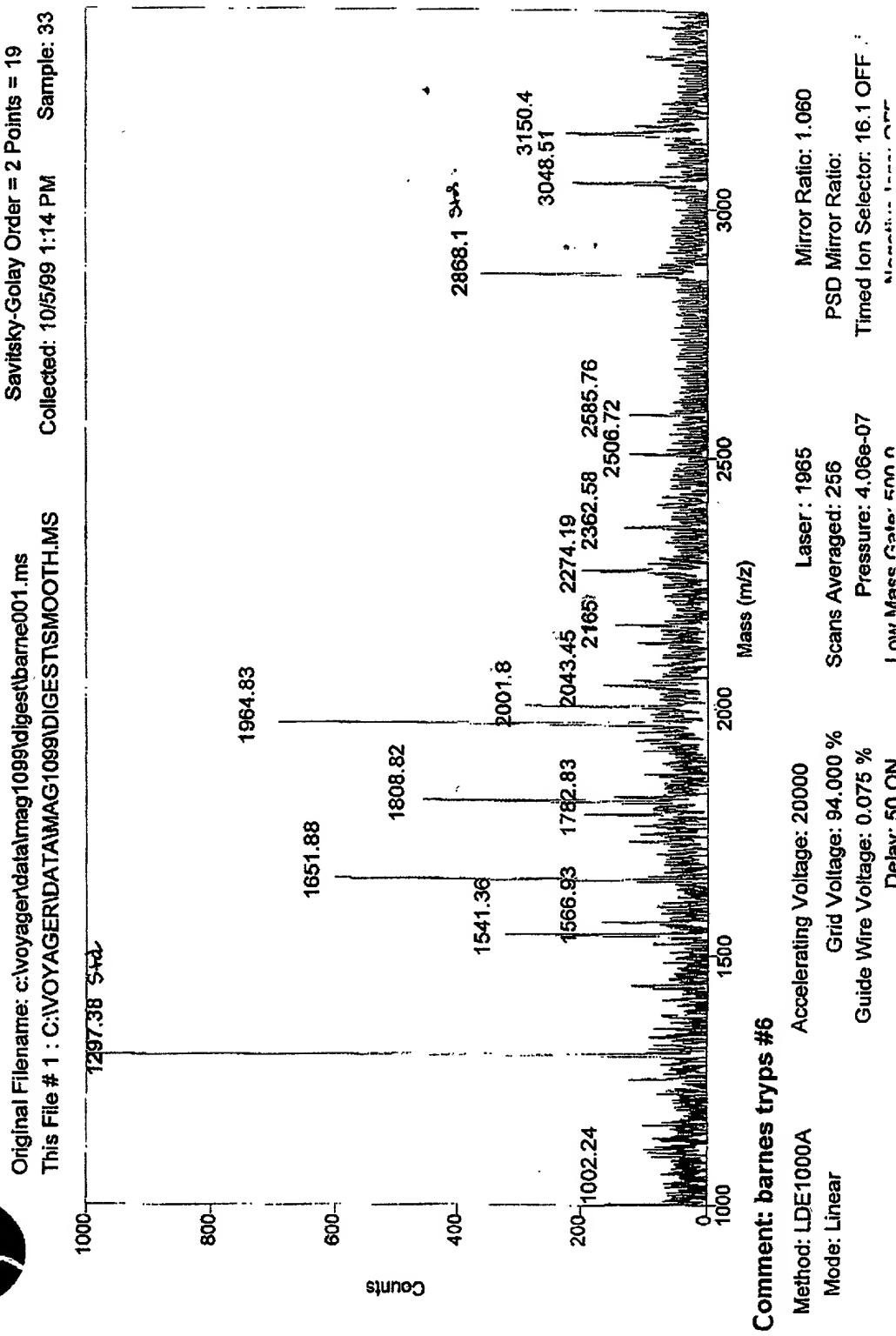


Figure 7F (Band 6)

Columbia University /IHHMI Protein Core



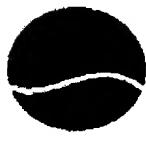


Figure 7G (Band 7)

Columbia University /HHMI Protein Core

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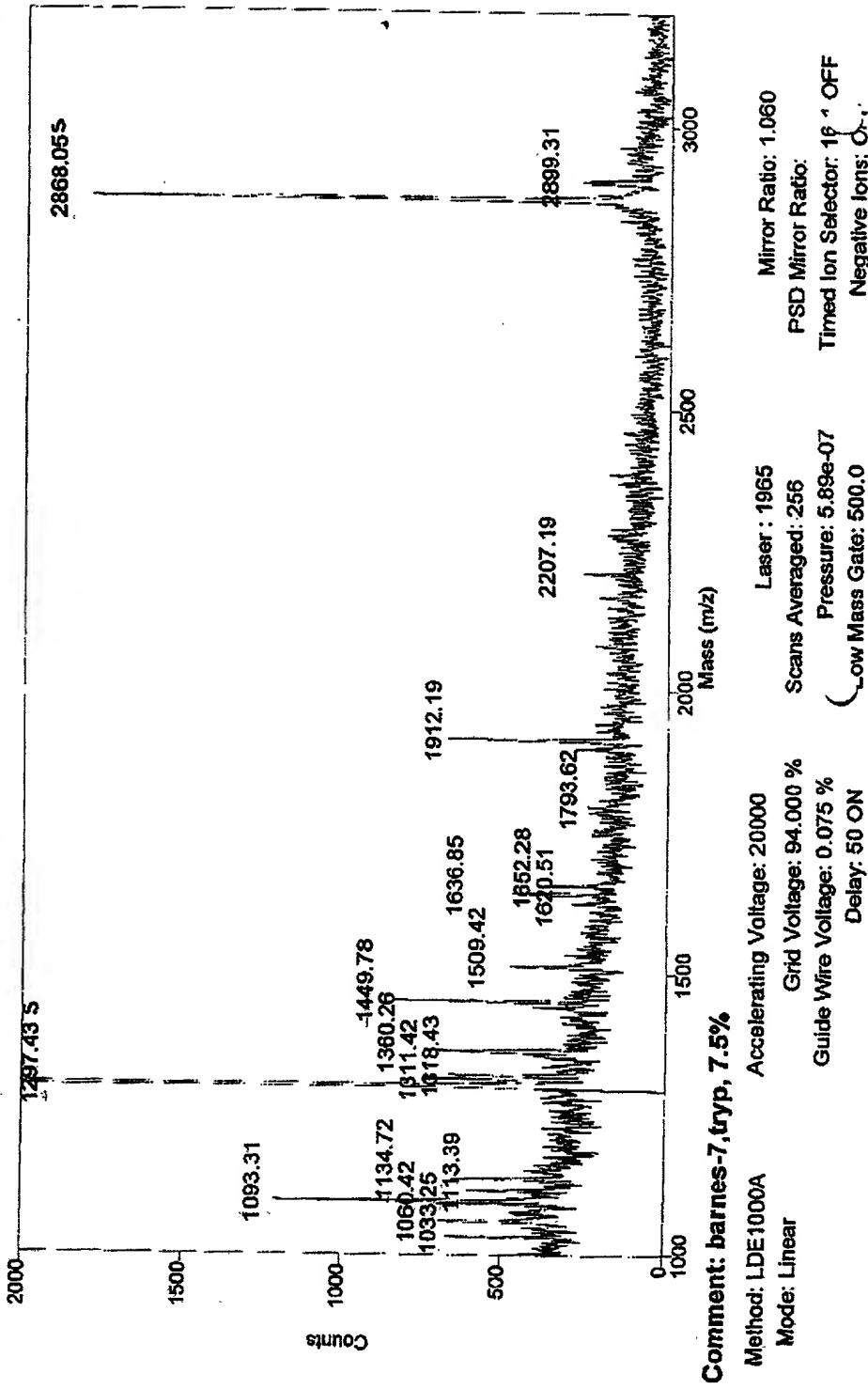


Figure 7H (Band 8)



Columbia University /HHMI Protein Core

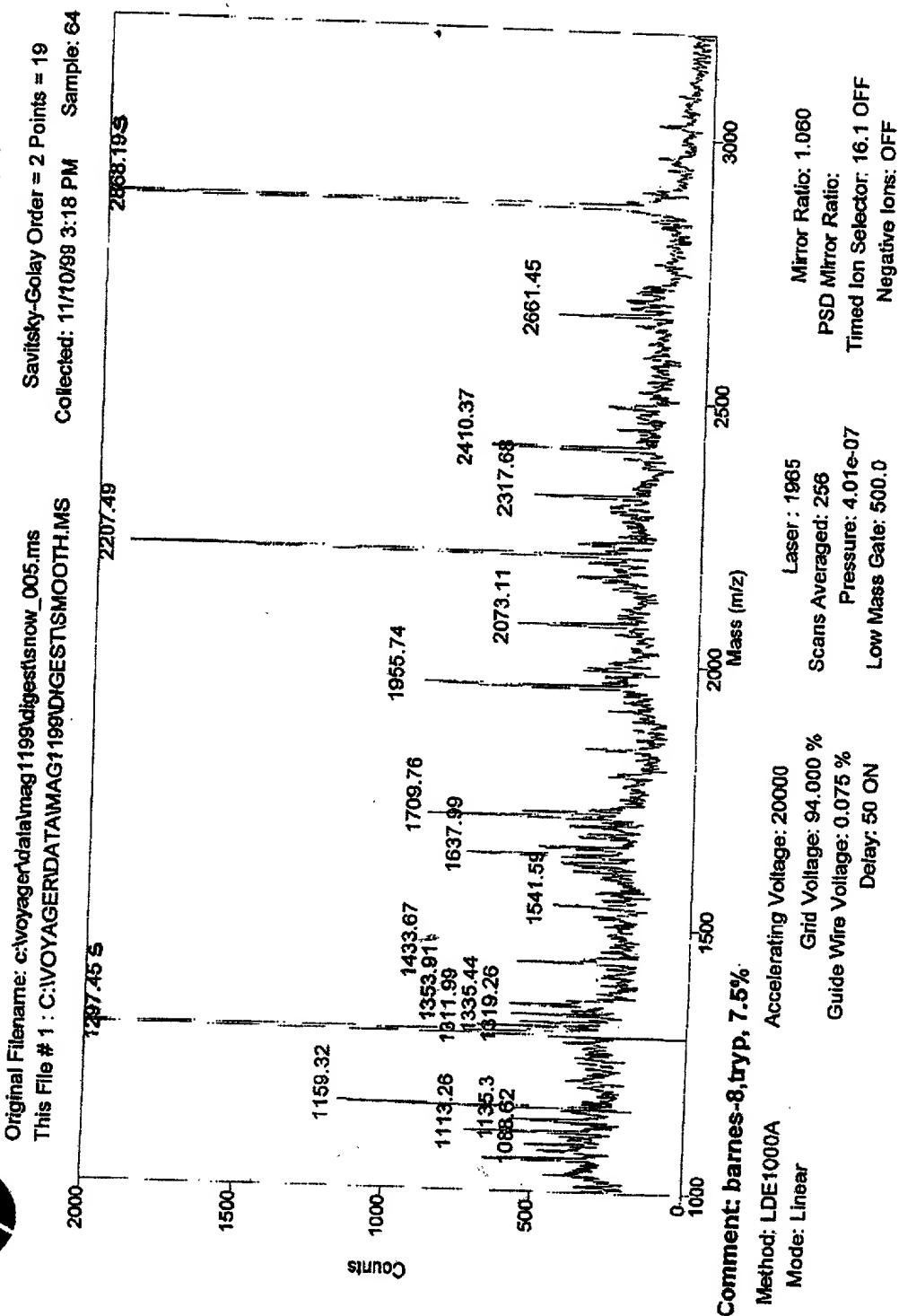


Figure 71 (Band 9)

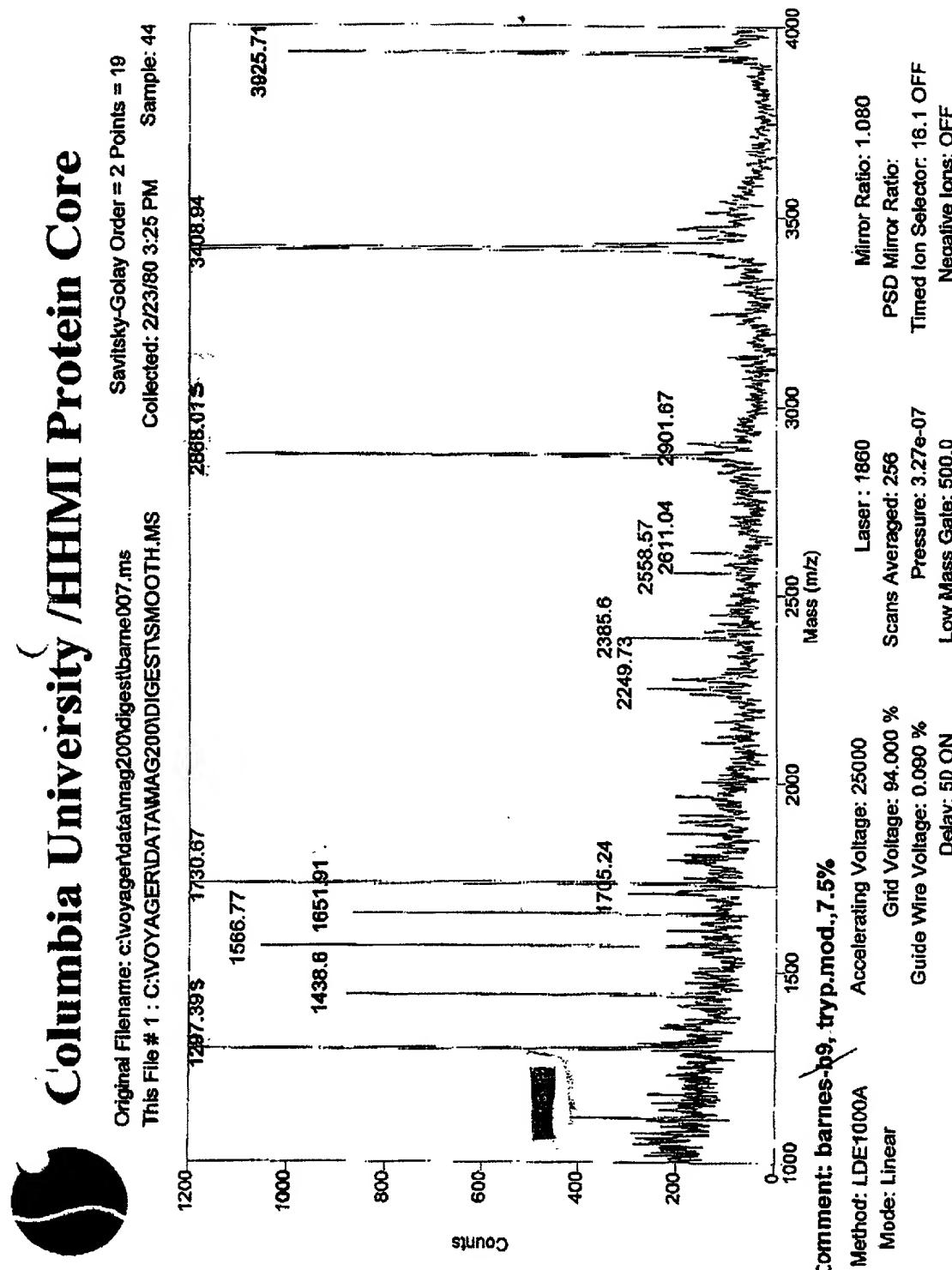


Figure 7J (Band 11)



Columbia University /HHMV Protein Core

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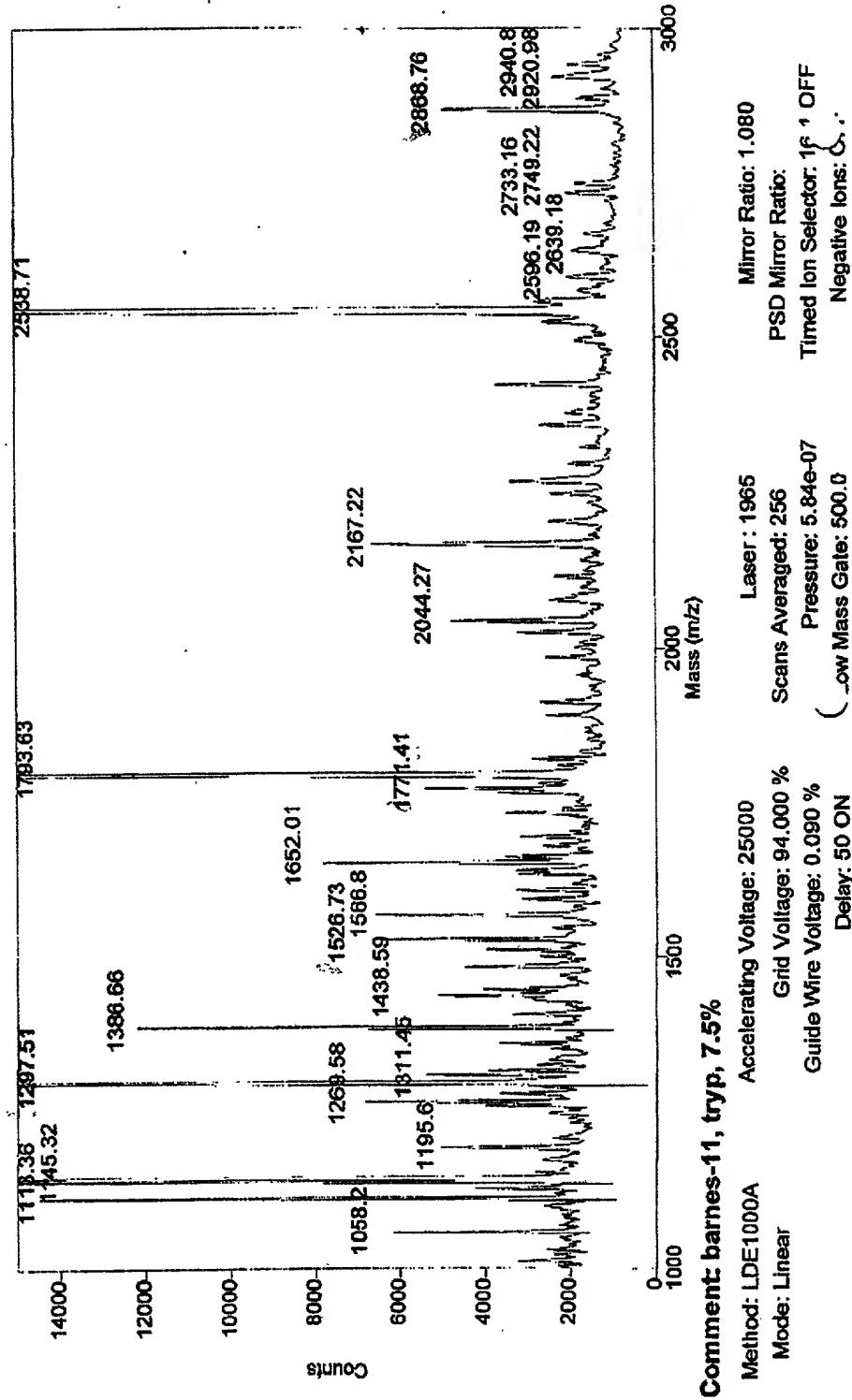
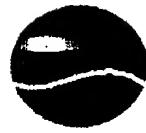


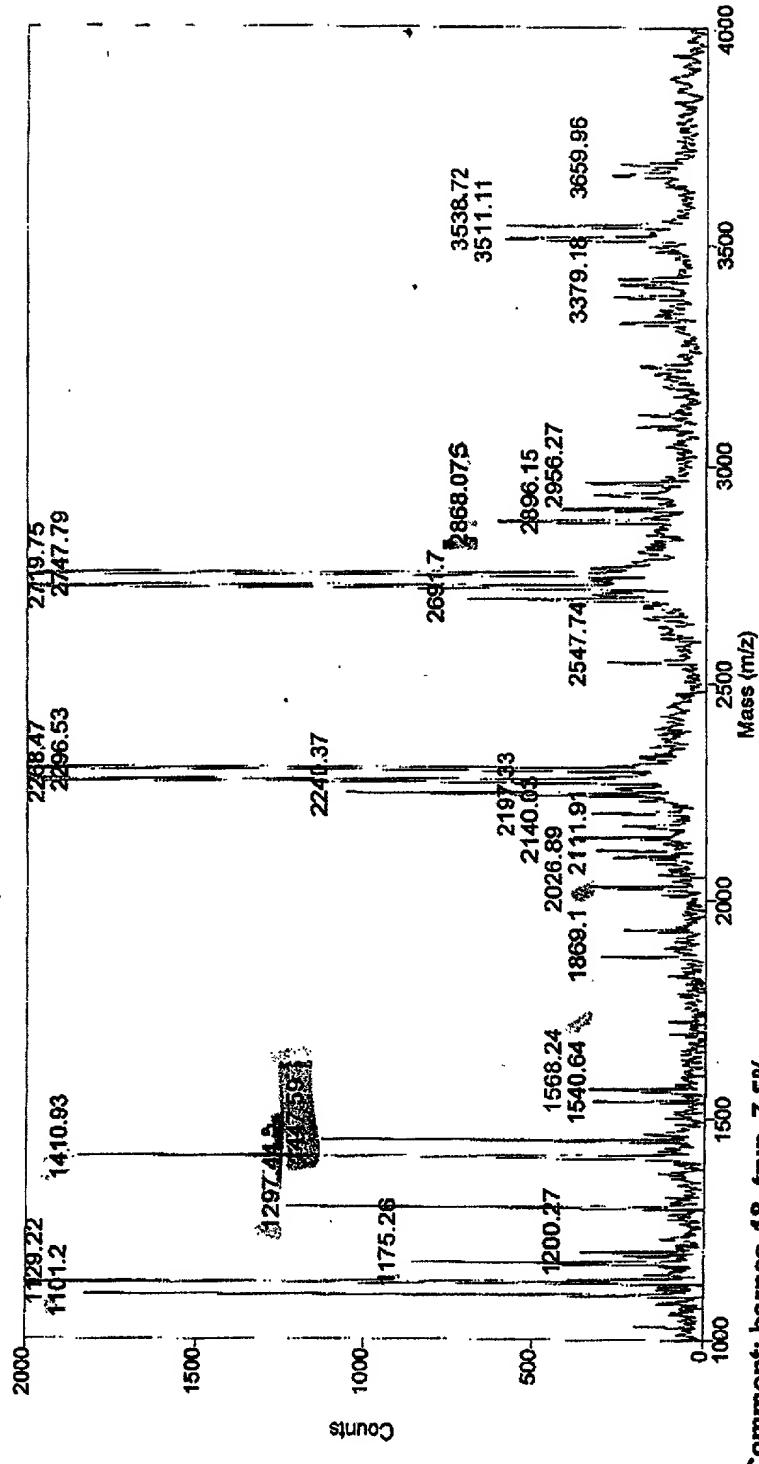
Figure 7K (Band 18)



Columbia University /HHMMI Protein Core

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Savitsky-Golay Order = 2 Points = 19
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Mode: Linear

Accelerating Voltage: 25000
Grid Voltage: 94.000 %
Guide Wire Voltage: 0.090 %
Delay: 50 ON

Laser: 1745
Scans Averaged: 256
Pressure: 2.900e-07
Low Mass Gate: 500.0

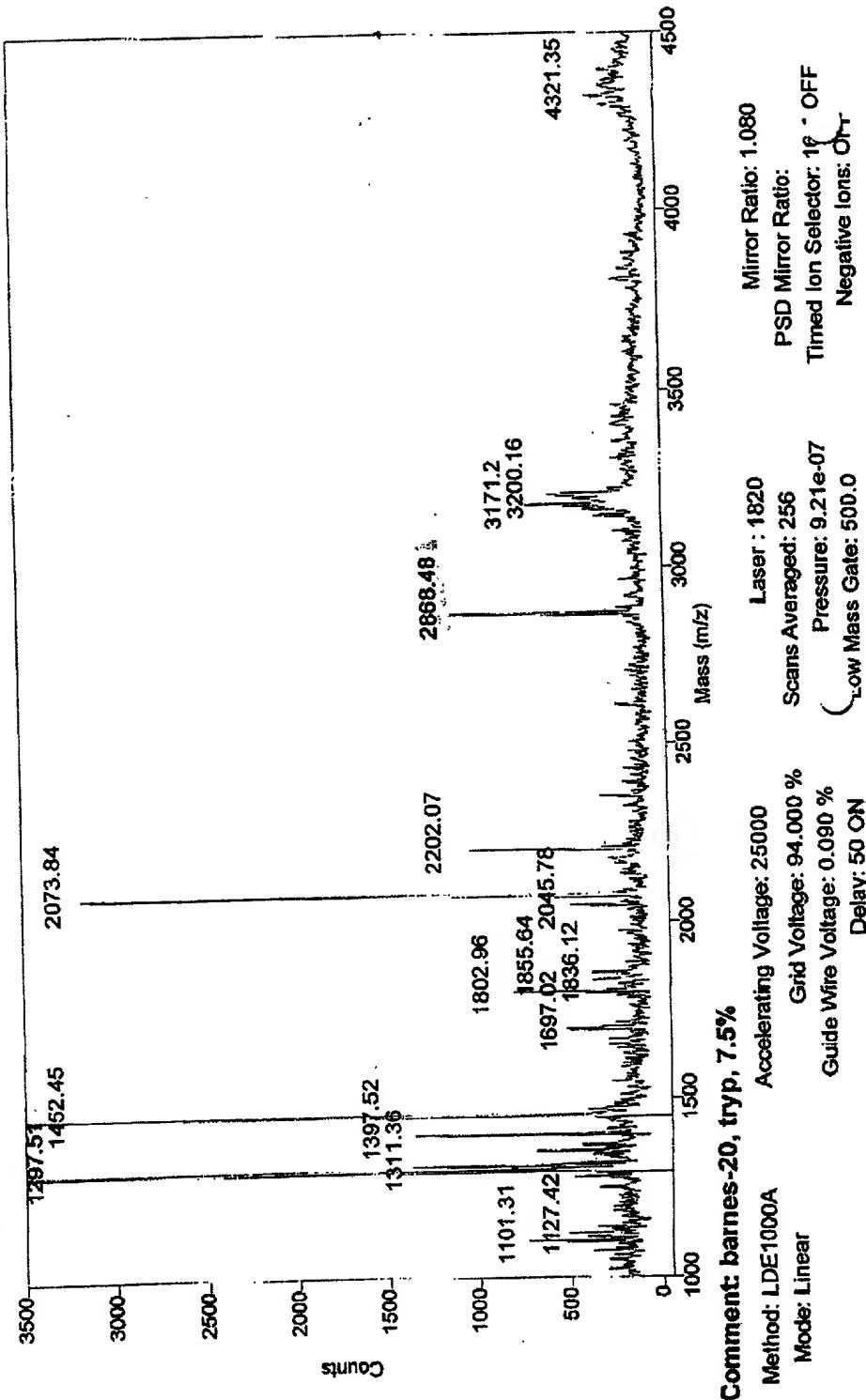
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Negative Ions: OFF

Figure 7L (Band 20)



Columbia University /HHMI Protein Core

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Columbia University /HHHMI Protein Core

Figure 7M (Band 22)

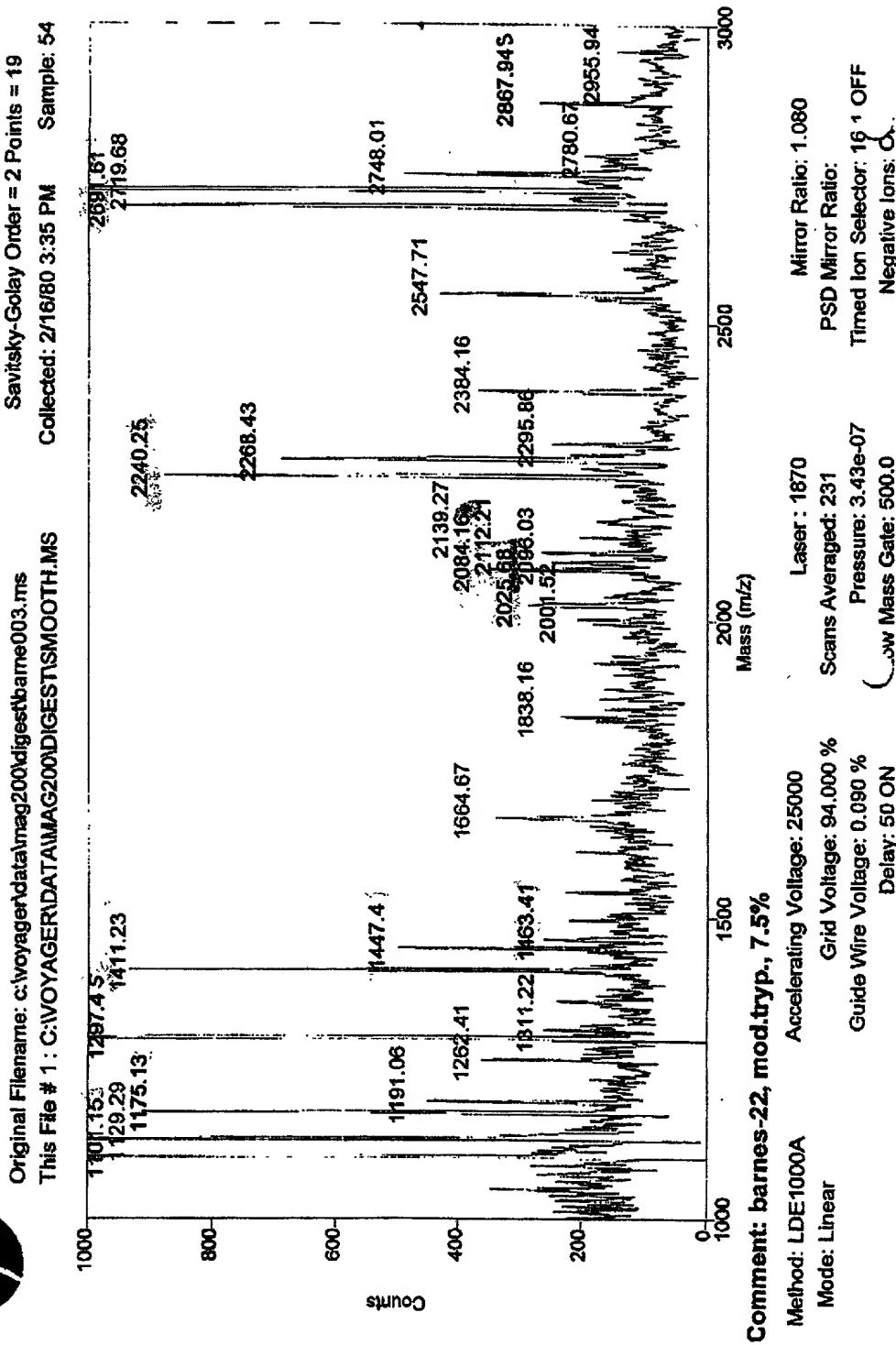




Figure 7N (Band 25)

Columbia University /HHMI Protein Core

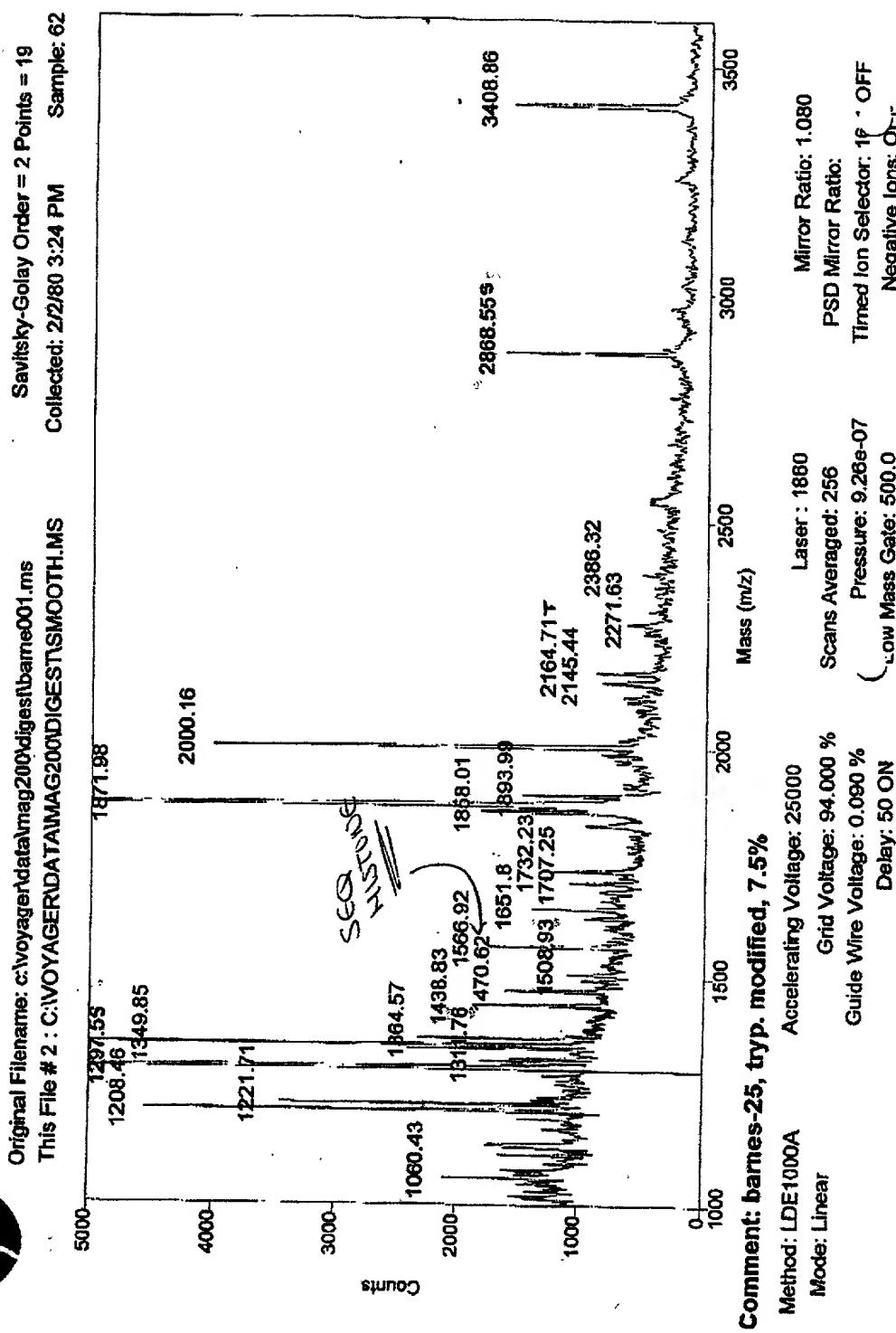




Figure 7O (Band 29)

Columbia University /HHMI Protein Core

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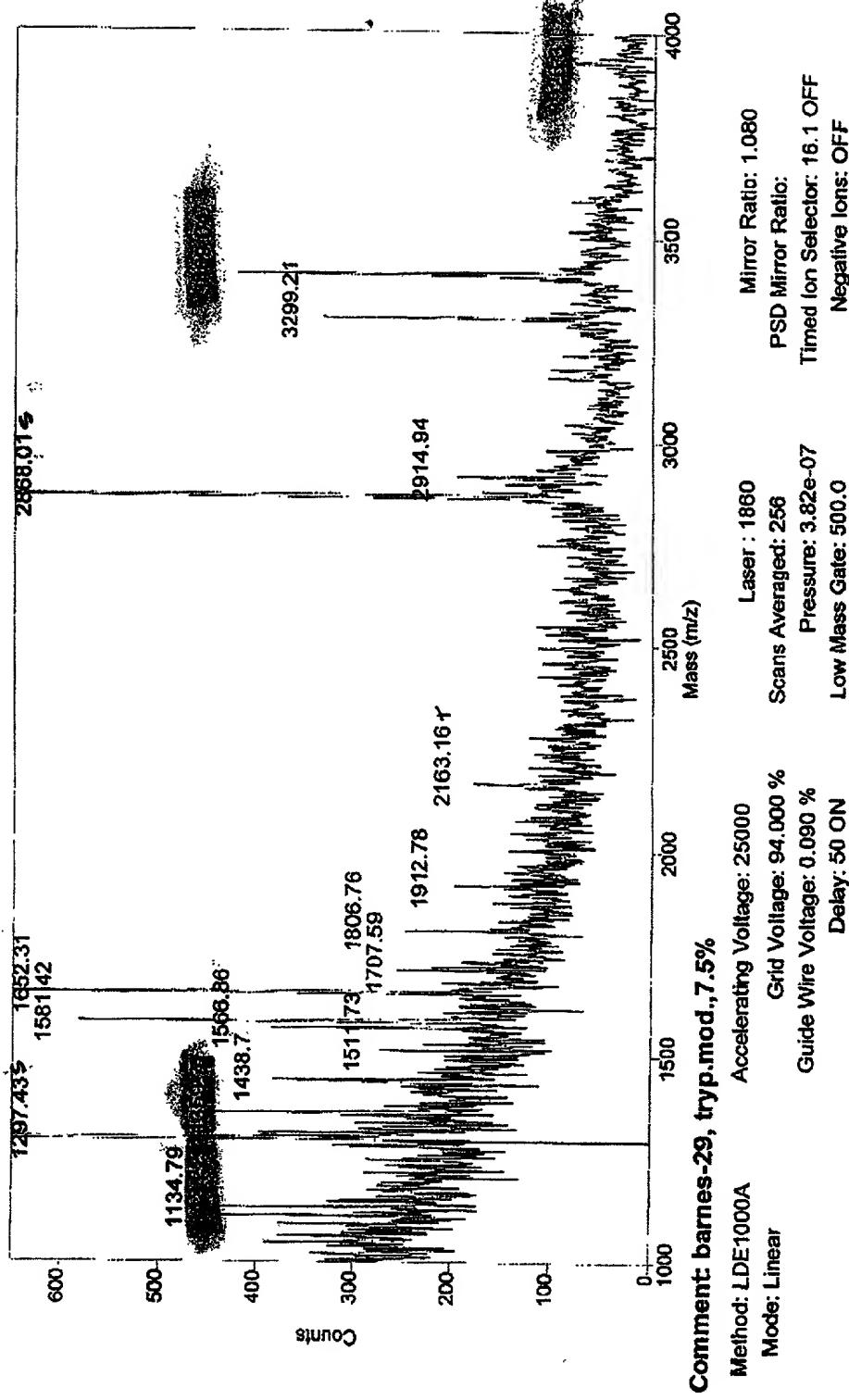


Figure 8



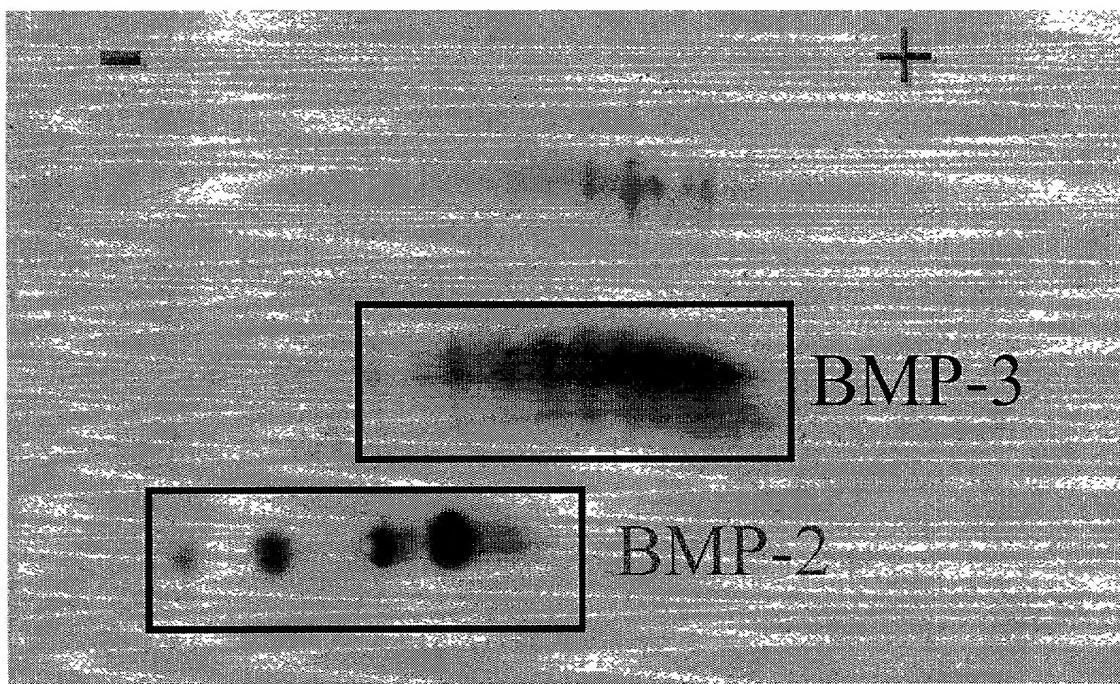


FIGURE 9A

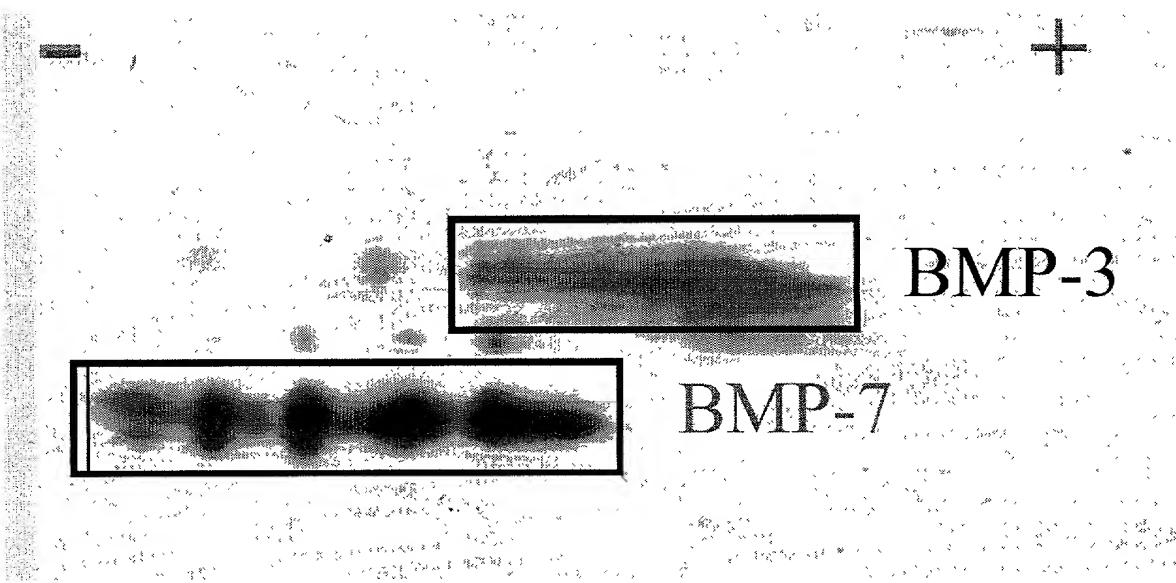


FIGURE 9B

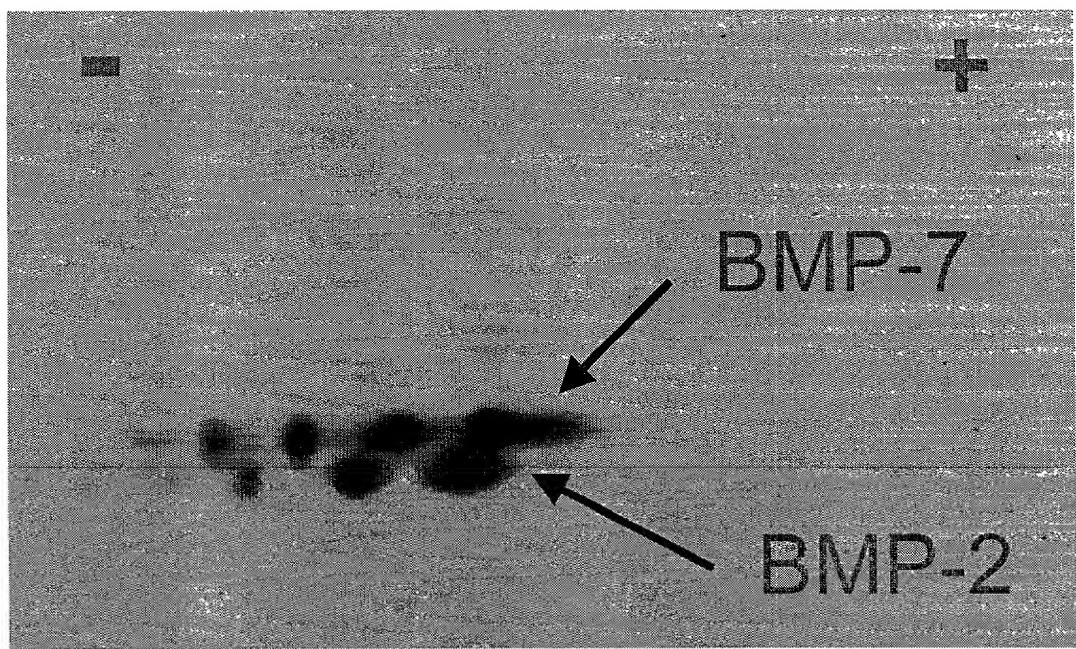


FIGURE 9C

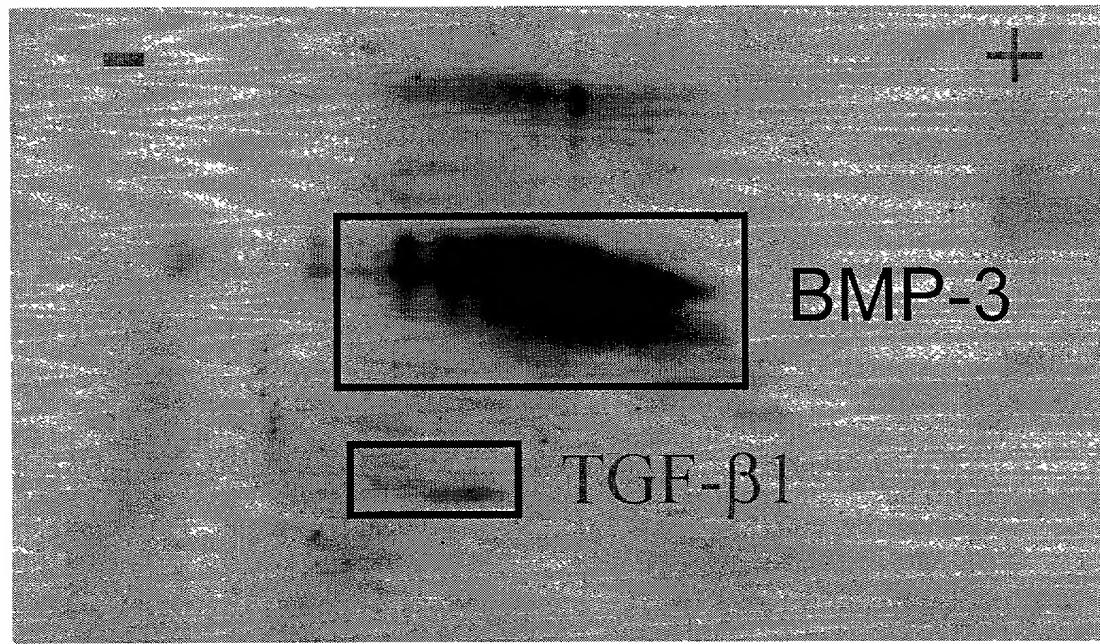
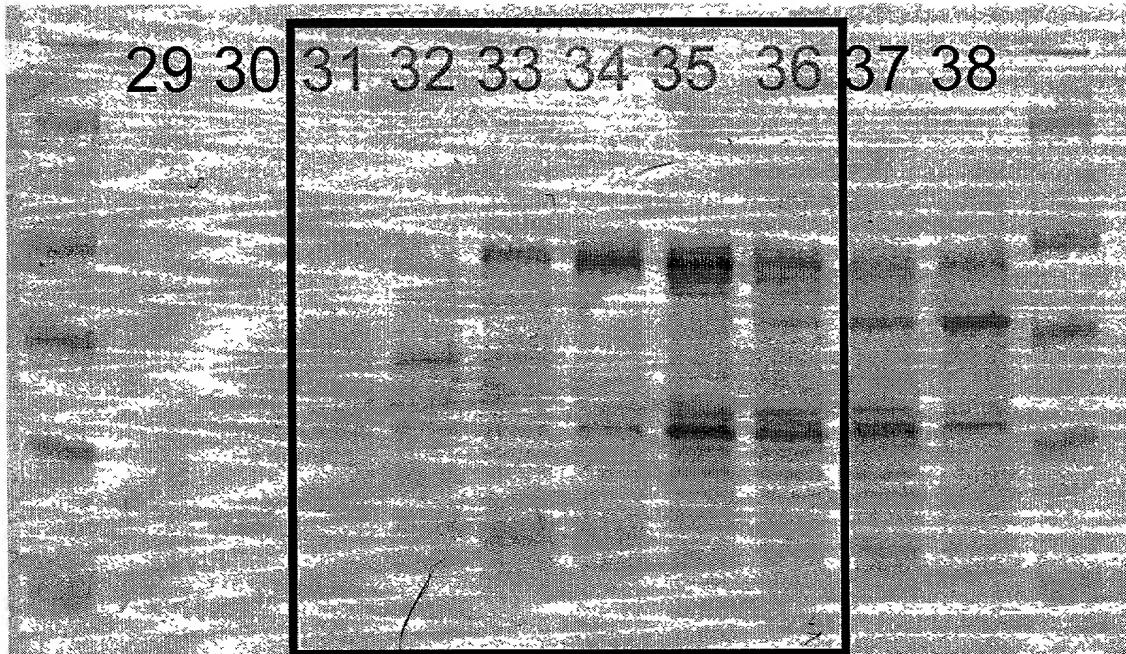


FIGURE 9D

FIGURE 10



- + +++ - + +++



FIGURE 11

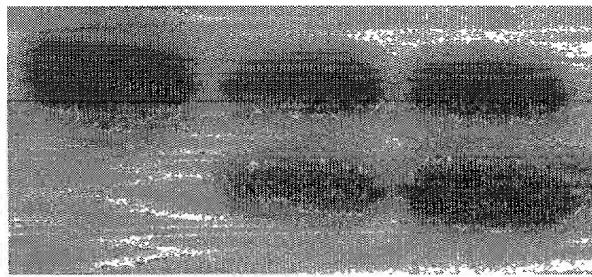


FIGURE 12

FIGURE 13A

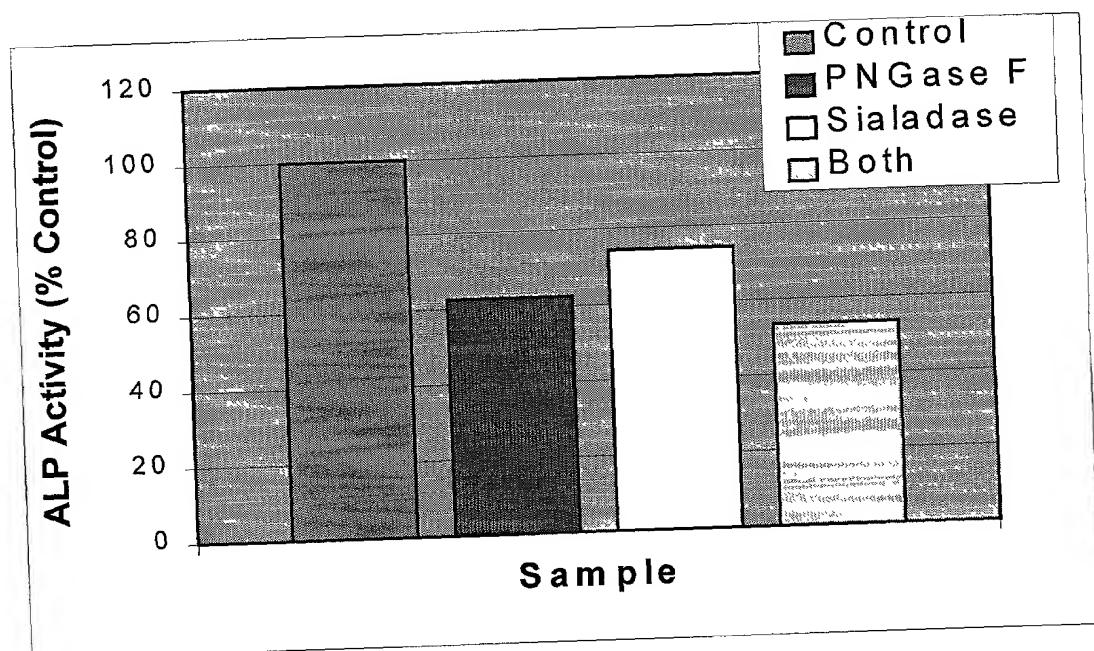
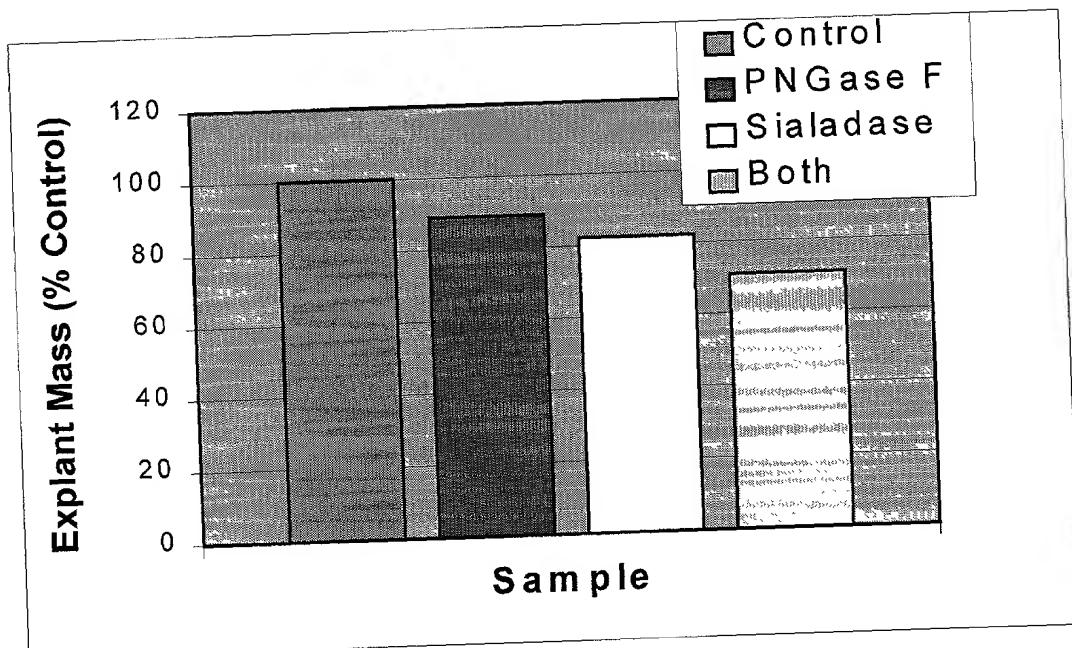


FIGURE 13B

FIGURE 14: Antibody Listing

Specificity	Antigen	Host Species	PC/MC	Source	Catalog No.
TGF- β 1 (human)	Protein	Rabbit	Polyclonal	Promega	G1221
TGF- β 2 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-90
TGF- β 3 (human)	Peptide	Rabbit	Polyclonal	Santa Cruz Biotechnology	sc-82
BMP-2 (human)	Protein	Rabbit	Polyclonal	Austral Biologics	PA-513-9
BMP-3 (human)	Peptide	Chicken	Polyclonal	Research Genetics	NA
BMP-4 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-6896
BMP-5 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-7405
BMP-6 (human)	Peptide	Mouse	Monoclonal	Novocastra Laboratories	NCL-BMP6
BMP-7 (human)	Peptide	Rabbit	Polyclonal	Research Genetics	NA
FGF-1 (human)	Peptide	Goat	Polyclonal	Santa Cruz Biotechnology	sc-1884
osteonectin (bovine)	Protein	Mouse	Monoclonal	DSHB	AON-1
osteocalcin (bovine)	Protein	Rabbit	Polyclonal	Accurate Chemicals	A761/R1H
serum albumin (bovine)	Protein	Rabbit	Polyclonal	Chemicon International	AB870
transferrin (human)	Protein	Chicken	Polyclonal	Chemicon International	AB797
apo-A1 lipoprotein (human)	Protein	Goat	Polyclonal	Chemicon International	AB740

Figure 15A: Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
1								
2	fx 49 (1579)	XLAAGAGYDVEK	ALAAAGAGYDVEK	11/11	histone H1.c	human	87668 (NCBI)	65-75
3	fx 67 (1346)	SLEKVCADLIR	SLEKVCADLIR	11/11	40s Ribosomal Protein S20	rat	R3RT20 (PIR)	31-41
4	fx 65 (0)	(V)YCGMLGFPSEAPV	(V)YCGMLGFPGEKRV	11/14	LORP	mouse	AAC95338 (NCBI)	213-226
5	N terminal seq	STGVLLPLQNNELPG	STGVLLPLQNNELPG	15/15	BMP-3	human	4557371 (NCBI)	290-304
5	fx 72 (3925)	STGVLLPLQNNELPGAEYQY	STGVLLPLQNNELPGAEYQY	20/20	BMP-3	human	4557371 (NCBI)	290-309
fx 74 (3409)	STGVLLPLQ	STGVLLPLQ	9/9	BMP-3	human	4557371 (NCBI)	290-298	
6	fx 55 (1566)	(SQTLQF)E	SQTLQFDE	7/8	BMP-3	human	4557371 (NCBI)	346-353
fx 47	VYAF	no match	???					
N terminal seq	HAGKYSREKNQPKP	HAGKYSREKNQPKP	11/14	α 2-Macroglobulin Receptor Assoc. Pro.	human	P30533 (Swiss-Prot)	31-46	
fx 57 (1438)	SQTLQFDEQ	SQTLQFDEQ	9/9	BMP-3	human	4557371 (NCBI)	346-354	
fx 57 (1652)	SLKPSNHA	SLKPSNHA	8/8	BMP-3	human	4557371 (NCBI)	410-417	
fx 51 (1093)	AALRPLVKP	AALRPLVKP	9/9	60s Ribosomal Protein L32	mouse	P179332 (Swiss-Prot)	1-9	
fx 37 (no M/S)	A(H)Q)QVERYYV	AVER	5/5	60s Ribosomal Protein L32	mouse	P179332 (Swiss-Prot)	109-113	
fx 37 (no M/S)	A(H)Q)QVERYYV	HQSDRYYV	5/7	60s Ribosomal Protein L32	mouse	P179332 (Swiss-Prot)	22-28	
8	fx 78 (0)	XALF(G)AQLGXALGPI	no match	???				
9	fx 56 (1567)	SQTLQFDEQT	SQTLQFDEQT	10/10	BMP-3	human	P12645 (Swiss-Prot)	346-355

Figure 15B: Identification of Proteins by Amino Acid Sequencing of Tryptic Fragments from 1D Gels

Band	Sample	Sequence Data	Best Database Match	Match	Identification	Species	Accession No.	AAs
11	fx 55 (131)	SQTLQF	SQTLQF	5/6	BMP-3	human	4557371 (NCBI)	346-351
	fx 47 (177)	VLATVTKPVGGDK	VLATVTKPVGGDK	13/13	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	87-99
	fx 76 (179)	XVFAL	XVFAL	4/4	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	273-276
	fx 61 (114)	AVPQLQGYLR	AVPQLQGYLR	9/10	60s Ribosomal Protein L6	human	Q02878 (Swiss-Prot)	262-271
18								
22	fx 58 (110)	ALDAAYCFR	ALDAAYCFR	9/9	TGF- β 2	human	P08112 (Swiss-Prot)	303-311
	fx 69 (no match)	GYNANFCAGACPYL	GYNANFCAGACPYL	14/14	TGF- β 2	human	P08112 (Swiss-Prot)	340-353
	fx 66 (1411.71)	VNSQSQLSPY	VNSQSQLSPY	9/9	SPP24	bovine	Q27967 (Swiss-Prot)	42-50
25	fx 39 (1470)	KAAKPSV(P)	KAAKPSV(P)	8/8	Histone H1 x	human	JC4928 (PIR)	199-206
29								

fx=fraction number (molecular weight of fragment, as measured by SDS-PAGE)

Figure 16A: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
1	4 peaks match with histone H1.c	human	876668 (NCBI)	1172.97 1579.87 1708.47 2011.58	1172.37 1579.71 1707.89 2012.32	0.60 0.16 0.58 -0.74	110-121 65-79 64-79 35-54	22	15 MS peaks match with Band 2
2	3 peaks match with histone H1 C	human	876668 (NCBI)	1579.76 1708.02 2012.12	1579.71 1707.89 2012.32	0.05 0.13 -0.20	65-79* 64-79 35-54	16	identification of starred peptide confirmed by sequence analysis
3	7 peaks match with ribosome S20	rat	R3RT20 (PIR)	1129.76 1156.21 1334.46 1352.13 1518.04 1919.02 3404.02	1129.40 1156.30 1334.62 1351.58 1517.77 1919.19 3404.87	0.36 -0.09 -0.16 0.55 0.27 -0.17 -0.85	50-59 76-83 56-66 88-99 9-21 5-21 88-119	62	15 MS peaks match with Band 1
4	3 peaks match with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	1987.95 2410.35 2610.57	1988.27 2410.63 2610.10	-0.32 -0.28 0.47	150-167 648-669 455-478	6	12 MS peaks match with Band 8

Figure 16B: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from 1D Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
5	9 peaks match with BMP-3	human	4557371 (NCBI)	1113.32	1113.31	0.01	361-368	48	% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)
				1438.53	1438.58	-0.05	346-357		
				1566.76	1566.76	0.00	345-357		
				1651.86	1651.91	-0.05	410-424		
				1794.09	1794.02	0.07	346-360		
				2268.46	2268.63	-0.17	374-392		
				2224.45	2424.81	-0.36	373-392		
				3409.15	3407.77	1.38	290-318*		
								17	
6	3 peaks match with α 2-Macroglobulin RAP	human	P30533 (Swiss-Prot)	1002.24	1002.15	0.09	283-290		Identification of starred peptide confirmed by sequence analysis
				2362.58	2362.43	0.15	129-150		
				3048.51	3048.52	-0.01	257-282		
				1566.93	1566.75	0.18	346-357	15	
2 peaks match with BMP-3	human	4557371 (NCBI)	1651.88	1651.91		-0.03	410-424		% coverage calculation is relative to the mature BMP-3, 183 AAS (290-472)

Figure 16C: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
7	4 peaks match with ribosome L32	mouse	P17932 (Swiss-Prot)	1033.25	1033.17	0.08	67-75	33	
				1033.31	1033.40	-0.09	1-10*		
8	5 peaks match with BMP-3	human	4557371 (NCBI)	1134.72	1134.28	0.44	65-74	21	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1449.78	1449.66	0.12	19-29		
9	1 peak matches with Lysyl Oxidase RP	human	NP002309 (Swiss-Prot)	1050.42	1060.20	0.22	102-111	3	12 MS peaks match with Band 4
				1113.39	1113.31	0.08	361-368		
	6 peaks match with BMP-3	human	4557371 (NCBI)	1360.26	1360.58	-0.32	190-200	36	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
				1652.28	1651.91	0.37	410-424		
				1793.62	1794.02	-0.40	346-360	36	
				2410.37	2410.63	-0.26	648-669		

Figure 16D: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
11	5 peaks match with BMP-3	human	4557371 (NCBI)	1113.23 1651.73 1793.58 2424.24 3408.34	1113.31 1651.91 1794.02 2424.81 3407.77	-0.08 -0.18 -0.44 -0.57 0.57	361-368 410-424 346-360 373-392 290-318	48	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)
			Q02878 (Swiss-Prot)	1140.38	1140.23	0.15	114-122	16	
			P47911 (Swiss-Prot)	1526.88 1059.15	1526.86 1059.12	0.02 0.03	141-155 10-20		
			P08112 (Swiss-Prot)	1145.36 1386.74	1145.35 1386.68	0.01 0.06	262-271 260-271		
			TGF- β 2	1101.20 1175.26 2240.37	1101.26 1175.42 2240.60	-0.06 -0.16 -0.23	303-311 400-409 312-328	52	
18	4 peaks match with TGF- β 2	human	Q27967 (Swiss-Prot)	2691.70	2691.91	-0.21	340-362		
				1410.93	1411.60	-0.67	42-53	30	
				1447.59	1447.65	-0.06	113-124		
				1540.64	1540.60	0.04	86-98		
				1869.10	1869.05	0.05	62-77		
5	5 peaks match with SPP24	bovine	2268.47	2268.57	-0.10	33-53			

Figure 16E: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
22	5 peaks match with TGF- β 2	human	P081122 (Swiss-Prot)	1101.15	1101.26	-0.11	303-311	63	
				1175.13	1175.42	-0.29	400-409		
				2084.16	2084.42	-0.26	312-347		
				2240.25	2240.60	-0.35	312-328		
				2691.61	2691.91	-0.30	340-362		
25	2 peaks match with SPP24	bovine	Q27967 (Swiss-Prot)	1411.23	1411.60	-0.37	42-53	11	
				1447.40	1447.65	-0.25	113-124		
				1208.46	1208.40	0.06	48-57		
25	5 peaks match with histone H1.x	human	JC4928 (PIR)	1221.71	1222.35	-0.64	107-118	14	
				1349.85	1350.52	-0.67	107-119		
				1364.57	1364.59	-0.02	48-58		
				1732.23	1732.97	-0.74	43-57		
				1060.43	1060.20	0.23	102-111		
25	5 peaks match with BMP-3	human	4557371 (NCBI)	1438.83	1438.58	0.25	346-357	31	% coverage calculation is relative to the mature BMP-3, 183 AAs (280-472)
				1566.92	1566.76	0.16	345-357		
				1651.80	1651.91	-0.11	410-424		
				3408.86	3407.77	1.09	290-318		

Figure 16F: Identification of Proteins by Mass Spectrometry of Tryptic Fragments from ID Gels

Band	Mass Spec Profile	Species	Accession Number	Mass Spec Data	Mass Spec Database	Mass Difference	AAs	% Coverage	Comments
29	4 peaks match with BMP-3	human	4557371 (NCBI)	1113.22 1438.70 1566.86 3409.04	1113.31 1438.58 1566.75 3407.77	-0.09 0.12 0.11 1.27	361-368 346-357 345-357 290-318	27	% coverage calculation is relative to the mature BMP-3, 183 AAs (290-472)

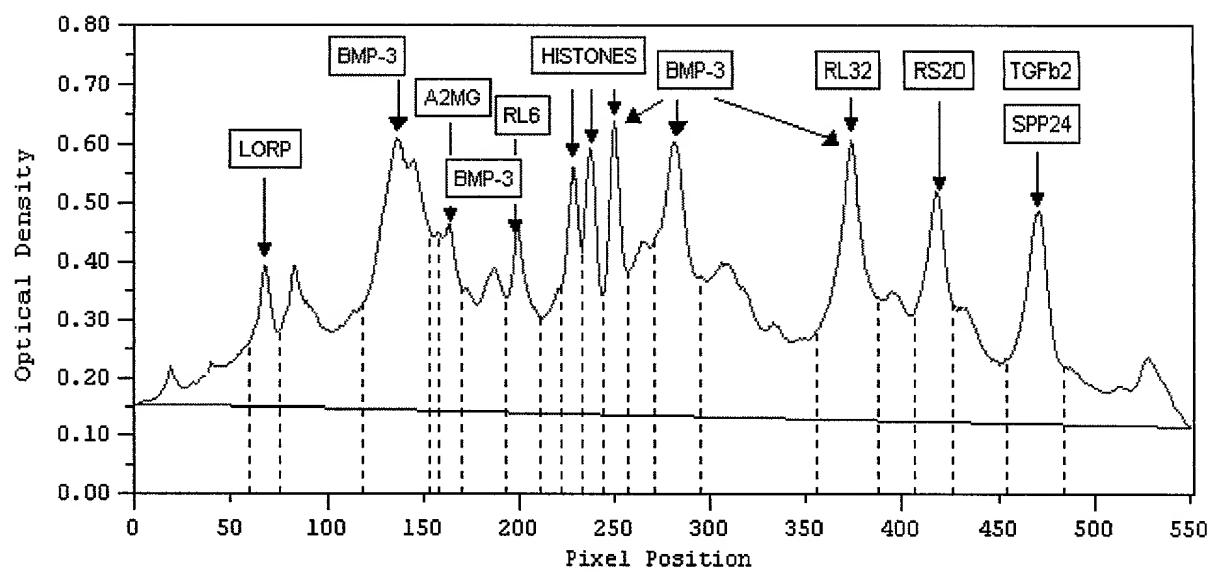


Figure 17A

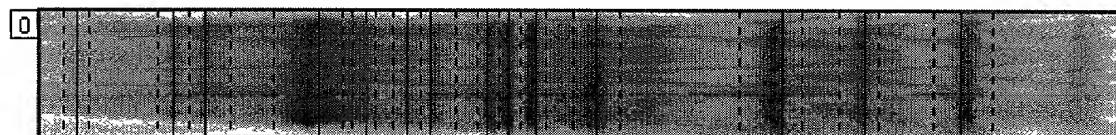


Figure 17B

FIGURE 18: Quantitation of Identified BP proteins

Identified Protein	Percentage of Total Protein
LORP	2
BMP-3	11
BMP-3 and A2-MG	3
RL6 & BMP-3	4
Histone	3
Histone	3
Histone & BMP-3	4
BMP-3	8
RL32 & BMP-3	8
RS2D	5
SPP24 & TGF- β 2	6
Total	58%

Figure 19A

Identification of Precisely Mass Spectrometry of Fragments from 2D Gels

Spp	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks Data	Database	Diff	AAS	% Coverage	Comments				
										MS Peaks Data	Database	Diff	AAS	% Coverage
1	Lys-C	2 peaks match with Coagulation Factor XIIIa	Human	P05180 (Swiss-Prote)	1837.01	1837.14	-0.13	472-497	5					
					1921.05	1921.14	0.51	364-382						
					2879.51	N/A	N/A	488-514						peptide match confirmed by sequence analysis
2	Trypsin	2 peaks match with LORP	Human	NP0022309 (Swiss-Prote)	1609.57	1609.65	-0.31	241-253	5					
					2410.60	2410.63	0.20	845-860						
3	Lys-C	8 peaks match with Cartilagin L. Precursor	Bovine	P25075 (Swiss-Prote)	1497.26	1496.60	0.40	105-116	41					
					1546.41	1546.70	0.14	59-70						
					1681.10	1660.80	0.36	21-33						
					1681.46	1680.80	1.06	301-314						
					1634.71	1634.00	0.71	310-314						
					2352.80	2351.50	1.40	274-285						
					2381.50	2380.70	0.80	219-261						
					2721.51	2721.10	0.41	131-154						

Figure 19C
Identification of Proteins by Mass Spectrometry of Fractions from 2D Gels

Figure 19D
Identification of Proteins by Mass Spectrometry of Fragments from 2D Gels

Spot	Digest	Mass Spec Profile	Species	Acc. No.	MS Peaks			AAs	% Coverage	Comments
					Data	Database	Diff			
9	Trypsin	7 peaks match Swiss-Prot	Mouse	P97351 (Swiss-Prot)	9220.05	8221.10	-0.05	19-23	20	
					1218.20	1218.31	-0.01	152-161		
					1348.62	1348.49	0.13	151-161		
					1516.69	1516.69	0.00	174-185		
					1593.72	1593.82	-0.10	94-108		
					1719.91	1720.00	-0.09	194-212		
					1953.12	1953.16	-0.04	65-81		
10	Trypsin	4 peaks match Human	Human	P76550 (NCBI)	1327.75	1327.68	0.10	24-46	23	
					1579.70	1579.71	-0.01	65-78		
					1707.65	1707.89	-0.24	64-79		
					2147.17	2147.51	-0.36	1-21		
11	Trypsin	6 peaks match Human	Human	P12750 (Swiss-Prot)	1183.48	1183.33	0.10	230-239	23	
					1216.30	1216.39	0.00	134-144		
					1354.03	1353.61	0.42	230-241		
					1507.81	1507.88	0.12	198-210		
					1557.75	1557.98	-0.23	37-49		
					2140.34	2140.54	-0.24	221-239		
					2391.60	2391.90	-0.10	77-93		

Figure 20. Quail Chorioallantoic Membrane (CAM) Angiogenesis Assay

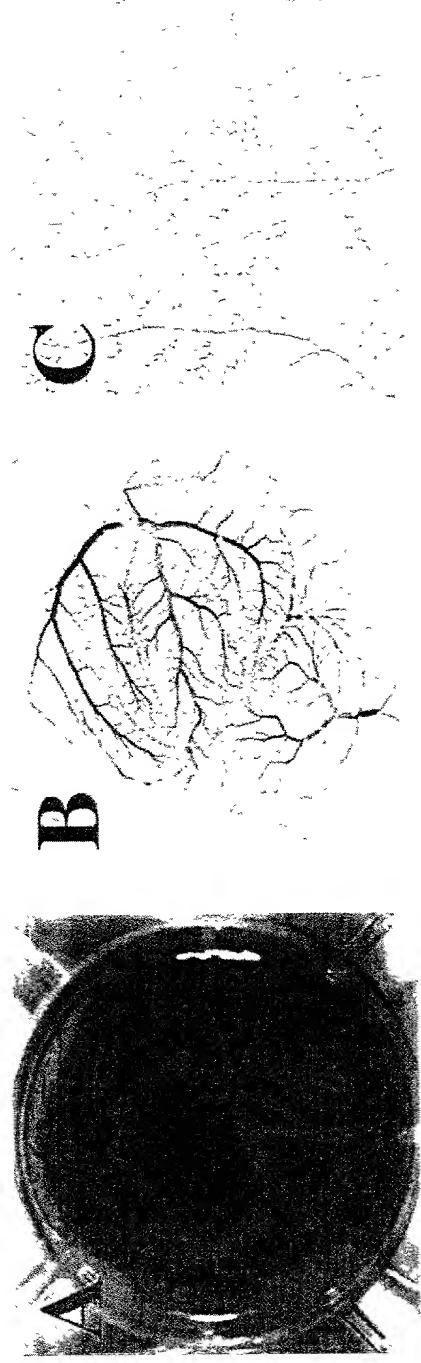


Figure 21. Black and white images of CAM vasculature after growth factor treatment

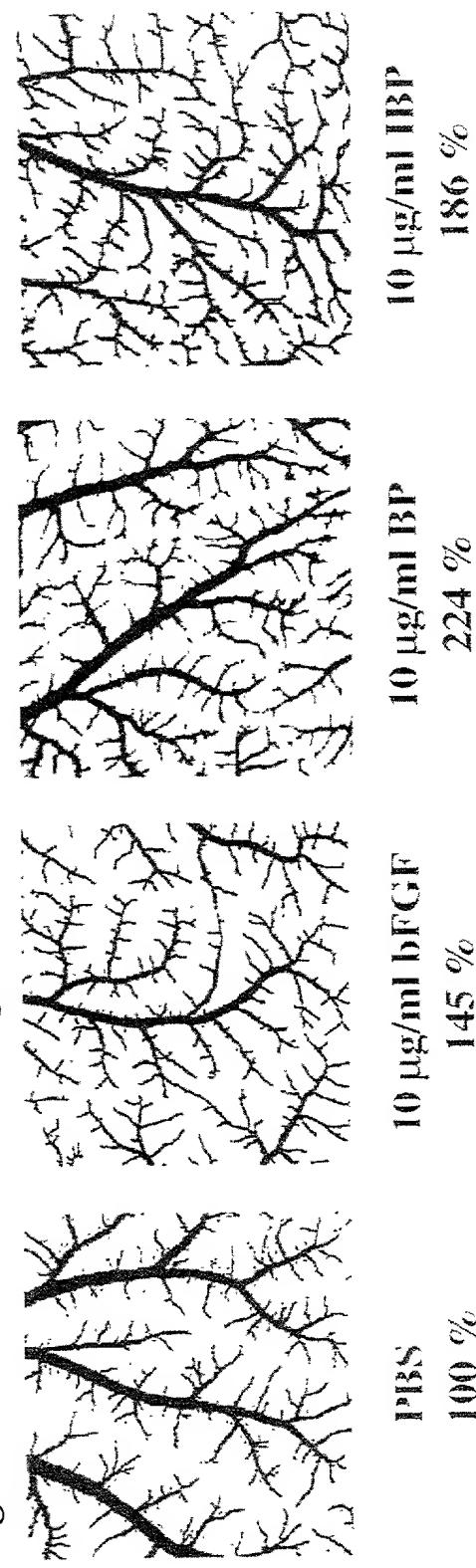
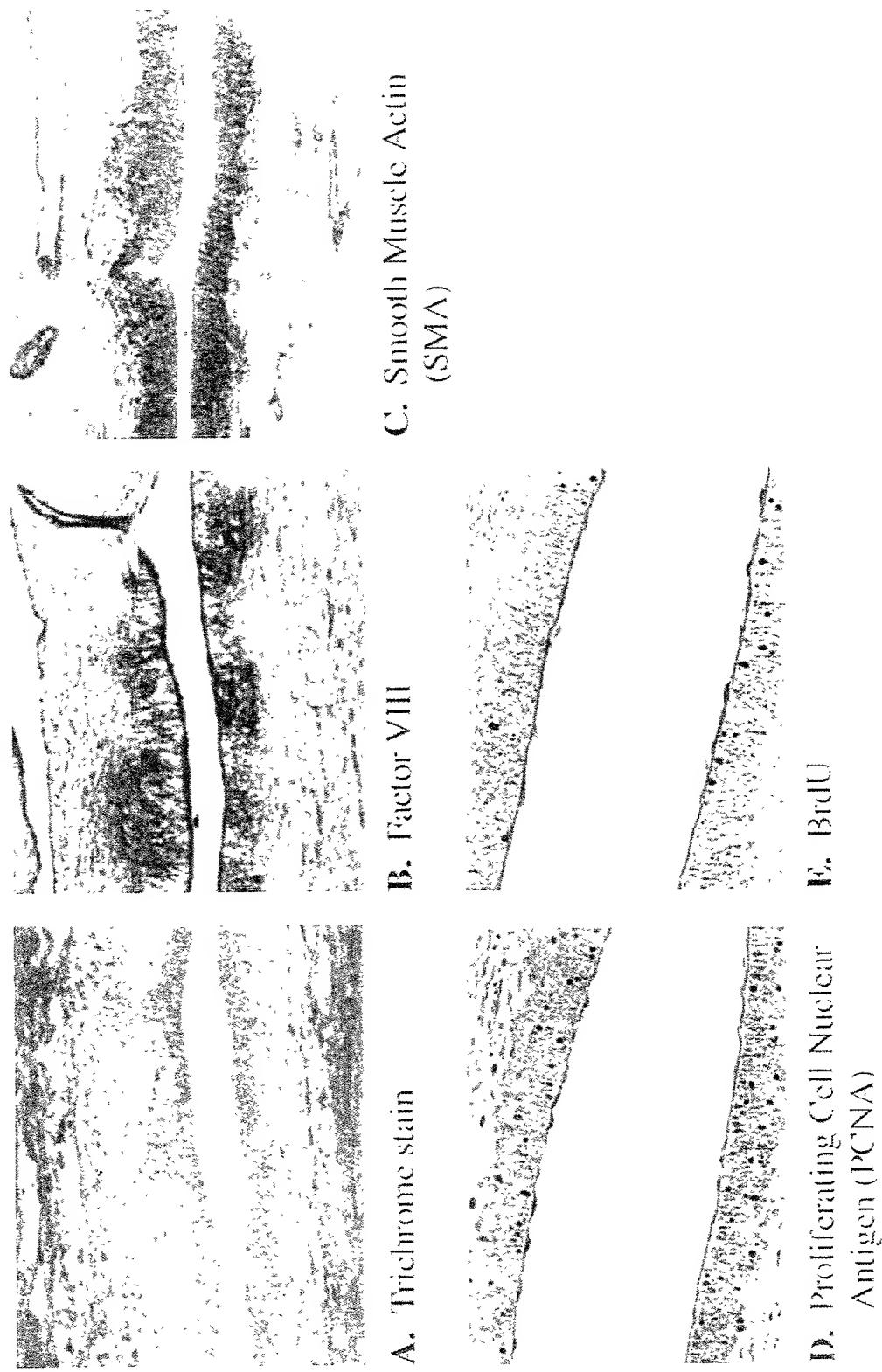


Figure 22. Histological sections of blood vessels formed in canine myocardium 2 weeks following BP injection



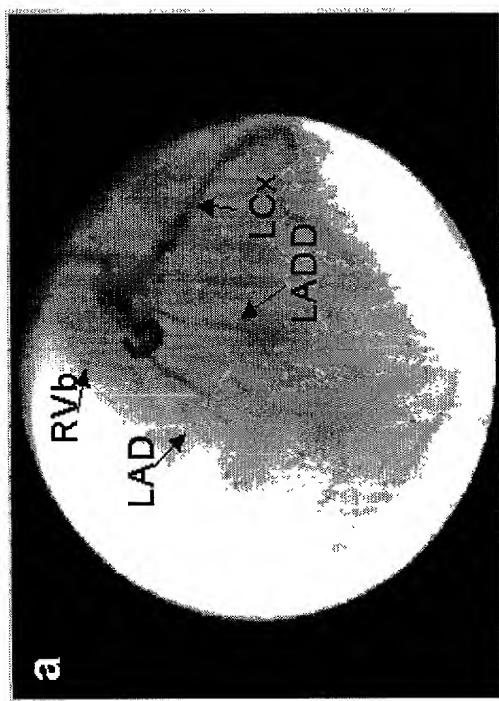


Fig. 23

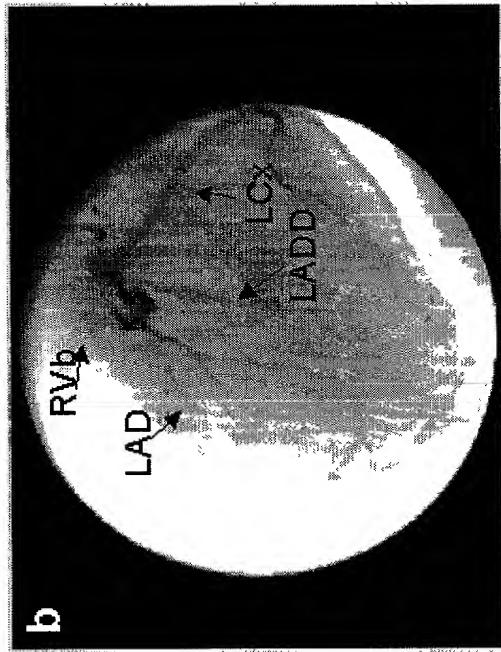


Fig. 24

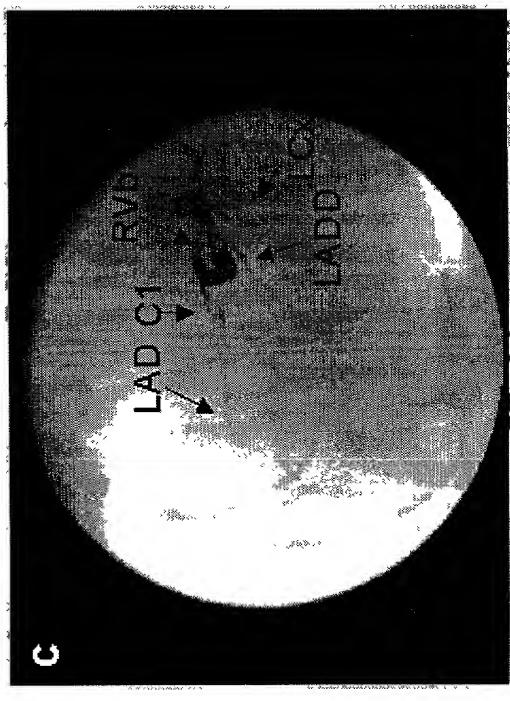


Fig. 25

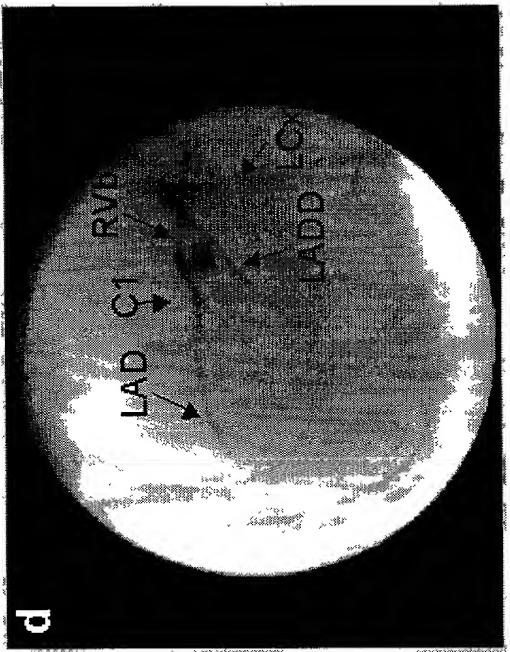


Fig. 26